```
=> d que stat l22
L8        26508 SEA FILE=HCAPLUS ABB=ON  BIOLOGICAL TRANSPORT+ALL AND CELL
                MEMBRANE+ALL
L10         736 SEA FILE=HCAPLUS ABB=ON  L8 AND (?HYDROPHOB? OR ?HYDROPHIL?)
L11          68 SEA FILE=HCAPLUS ABB=ON  L10 AND PH
L12           2 SEA FILE=HCAPLUS ABB=ON  L10 AND PH(3A)?SENSITIV?
L15           1 SEA FILE=HCAPLUS ABB=ON  L10 AND ?VINYL?
L16           5 SEA FILE=HCAPLUS ABB=ON  L11 AND (?ENDOSOM? OR ?ENDOCYT?)
L18           4 SEA FILE=HCAPLUS ABB=ON  L10 AND (?POLYALK?(W)?OXID? OR
                ?POLYOXYALKYLENE?)
L19          72 SEA FILE=HCAPLUS ABB=ON  L11 OR L12 OR L15 OR L16 OR L18
L20          45 SEA FILE=HCAPLUS ABB=ON  L19 AND (PRD<20000107 OR PD<20000107)
L21           1 SEA FILE=HCAPLUS ABB=ON  L20 AND (?THERAP? OR ?DIAG?)
L22          45 SEA FILE=HCAPLUS ABB=ON  L20 OR L21

=> d ibib abs l22 1-45

L22  ANSWER 1 OF 45  HCAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2000:783177  HCAPLUS
DOCUMENT NUMBER:          134:361246
TITLE:                    Partitioning equilibrium between uncharged and charged
                          local anesthetic lidocaine in the surface-adsorbed
                          film
AUTHOR(S):                Matsuki, Hitoshi; Kaneshina, Shoji; Kamaya, Hiroshi;
                          Ueda, Issaku
CORPORATE SOURCE:         Department of Biological Science and Technology, The
                          University of Tokushima, Tokushima, Japan
SOURCE:                   Masui to Sosei (2000), 36(1-2), 37-40
                          CODEN: MASODV; ISSN: 0385-1664
PUBLISHER:                Hiroshima Masui Igakkai
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   Partitioning equilibrium between uncharged local anesthetic lidocaine (LC) and
     charged one (LC • H+) in the surface-adsorbed film was investigated by
     measuring the surface tension and pH of aqueous solns. of uncharged
     anesthetic LC and hydrochloric acid (HCl) mixture  The values of surface
     tension decreased slightly with increasing mt (total molality) at
     0≤X2 (mole fraction of LC in the mixture)≤ 0.5 while they
     decreased with mt rapidly at 0.5 <X2≤ 1.  The results of  pH
     measurements showed that almost all LC were changed into LC • H+ by
     protonation at 0≤X2≤ 0.5 and LC and LC • H+ coexist only
     at 0.5<X2≤1.  We evaluated the quantities of resp. LC and LC •
     H+ transferred from the aqueous solution to the surface-adsorbed film, that is
     their surface densities, by analyzing the exptl. results
     thermodynamically.  We found that the partitioning behavior of LC and LC
     • H+ in the surface-adsorbed film is different in the three composition
     regions: (1) slight partitioning of low surface-active LC • H+ at
     0≤X2≤0.5 because of no existence of LC in the region, (2)
     preferential partitioning of LC than LC • H+ at 0.5<X2<.apprx.0.7, and
     (3) neg. partitioning of LC • H+ expelled from the surface region at
     high X2 range.  Taking into account that the physiol. pH value
     in vivo has a value of .apprx.7.4, LC partitions preferentially into the
     surface-adsorbed film rather than LC • H+ at the composition corresponding
     to the pH in this study.  Present results clearly suggest that
     uncharged local anesthetics transfer into hydrophobic
     environments such as cell membranes more easily than charged ones.

L22  ANSWER 2 OF 45  HCAPLUS  COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER:         2000:601475  HCAPLUS
DOCUMENT NUMBER:          133:291069
```

TITLE: The membrane insertion of trichosanthin is membrane-surface-**pH** dependent
AUTHOR(S): Xia, Xiao-Feng; Sui, Sen-Fang
CORPORATE SOURCE: State Key Laboratory of Biomembranes, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing, 100084, Peop. Rep. China
SOURCE: Biochemical Journal (**2000**), 349(3), 835-841
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Trichosanthin (TCS) is the active component extracted from Tianhuafen, a traditional herbal medicine that has been used for abortion in China for centuries. It belongs to the type-I ribosome-inactivating protein (RIP) family and can inactivate the eukaryotic ribosome through its RNA N-glycosidase activity. Recent studies have shown TCS to be multifunctional, its pharmacol. properties including immunomodulatory, anti-tumor and anti-HIV activities. The membrane-insertion property of TCS is thought to be essential for its physiol. effect, for it must get across the membrane before it can enter the cytoplasm and exert its RIP function. In this paper, the membrane-insertion mechanism of TCS was studied. The monolayer experiment revealed that TCS's membrane-insertion ability was dependent on low **pH**. Fluorescence spectroscopy using 1-anilinonaphthalene-8-sulfonic acid as a probe showed that low **pH** may induce the conformational change of TCS that leads to the **hydrophobic**-site exposure, and the CD result showed that this conformational change did not alter its secondary structure. Such conformational change leads to an intermediate state, called the "molten globular state" by previous investigators. The **pH**-dependent membrane insertion and conformational change were related by the fact that the optimal membrane-surface **pH** needed was the same for the two events. From these and other results, a membrane-insertion model was proposed.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 3 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:547374 HCAPLUS
DOCUMENT NUMBER: 133:155438
TITLE: Active agent transport systems comprising amino acids
INVENTOR(S): Milstein, Sam J.; Barantsevitch, Evgueni; Leone-Bay, Andrea; Wang, Nai Fang; Sarubbi, Donald J.; Santiago, Noemi B.
PATENT ASSIGNEE(S): Emisphere Technologies, Inc., USA
SOURCE: U.S., 71 pp., Cont.-in-part of U.S. 5,714,167.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 30
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6099856	A	20000808	US 1996-763183	19961210 <--
US 5443841	A	19950822	US 1992-920346	19920727 <--
US 5451410	A	19950919	US 1993-51019	19930422 <--
US 5578323	A	19961126	US 1993-76803	19930614 <--
US 5447728	A	19950905	US 1993-168776	19931216 <--
US 5792451	A	19980811	US 1994-205511	19940302 <--
WO 9423767	A1	19941027	WO 1994-US4560	19940422 <--

W: AT, AU, BB, BG, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

US 5541155	A	19960730	US 1994-231623	19940422	<--
US 5629020	A	19970513	US 1994-231622	19940422	<--
US 5693338	A	19971202	US 1994-315200	19940929	<--
US 6331318	B1	20011218	US 1994-316404	19940930	
ZA 9408342	A	19950622	ZA 1994-8342	19941024	<--
US 5714167	A	19980203	US 1994-328932	19941025	<--
US 6221367	B1	20010424	US 1997-939939	19970929	<--
WO 9825589	A1	19980618	WO 1997-US23545	19971209	<--

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9855322	A1	19980703	AU 1998-55322	19971209	<--
AU 771024	B2	20040311	AU 2000-72261	20001214	<--
AU 771434	B2	20040325	AU 2000-72260	20001214	<--
US 2001039258	A1	20011108	US 2001-760307	20010111	<--
US 2002155993	A1	20021024	US 2002-125836	20020419	<--
US 6663898	B2	20031216			
US 2003133953	A1	20030717	US 2002-255237	20020925	<--
US 6916489	B2	20050712			
US 2003198658	A1	20031023	US 2003-443713	20030521	<--
US 6906030	B2	20050614			
AU 2004202745	A1	20040923	AU 2004-202745	20040623	
US 2005255138	A1	20051117	US 2005-42960	20050124	<--
US 2005142156	A1	20050630	US 2005-61590	20050218	<--

PRIORITY APPLN. INFO.:

US 1992-898909	B2	19920615	<--
US 1992-920346	A2	19920727	<--
US 1993-51019	A2	19930422	<--
US 1993-76803	A2	19930614	<--
US 1993-143571	B2	19931026	<--
US 1993-168776	A2	19931216	<--
US 1994-205511	A2	19940302	<--
US 1994-231622	A2	19940422	<--
US 1994-231623	A2	19940422	<--
WO 1994-US4560	A2	19940422	<--
US 1994-315200	A2	19940929	<--
US 1994-316404	A2	19940930	<--
US 1994-328932	A2	19941025	<--
US 1996-17902P	P	19960329	<--
US 1996-763183	A2	19961210	<--
US 1997-820694	A2	19970318	<--
US 1997-939939	A1	19970929	<--
WO 1997-US23545	W	19971209	<--
AU 1998-62756	A3	19980206	<--
US 1999-420200	A1	19991018	<--
AU 2000-72260	A3	20001214	
US 2001-929530	A1	20010813	
US 2002-125836	A1	20020419	
US 2002-255237	A1	20020925	
US 2003-443713	A1	20030521	

OTHER SOURCE(S): MARPAT 133:155438

AB Methods for transporting a biol. active agent across a cellular membrane or a lipid bilayer. A first method includes the steps of: (a) providing a biol. active agent which can exist in a native conformational state, a denatured conformational state, and an intermediate conformational state which is reversible to the native state and which is conformationally between the native and denatured states; (b) exposing the biol. active agent to a complexing perturbant to reversibly transform the biol. active agent to the intermediate state and to form a transportable supramol. complex; and (c) exposing the membrane or bilayer to the supramol. complex, to transport the biol. active agent across the membrane or bilayer. The perturbant has a mol. weight between about 150 and about 600 Daltons, and contains at least one **hydrophilic** moiety and at least one **hydrophobic** moiety. The supramol. complex comprises the perturbant non-covalently bound or complexed with the biol. active agent. In the present invention, the biol. active agent does not form a microsphere after interacting with the perturbant. A method for preparing an orally administrable biol. active agent comprising steps (a) and (b) above is also provided as are oral delivery compns. Addnl., mimetics and methods for preparing mimetics are contemplated. Native gradient gels were run with 647 mg/mL of α-interferon, and increasing amts. (10-500 mg/mL) of perturbant (a mixture of L-Valine, L-Leucine, L-phenylalanine, L-lysine and L-arginine modified with benzenesulfonylchloride). As the amount of perturbant added was increased in each subsequent lane relative to a fixed concentration of α-interferon, the α-interferon migrated to a lower, rather than a higher, mol. weight This indicated that the α-interferon structure was changing, because if the structure was not changing, there would be a shift towards higher mol. weight as perturbant complexes with the active agent. Oral administration of above α-interferon and perturbant to rats at 500 μg/kg showed significant blood level of α-interferon as compared with controls with no perturbant.

REFERENCE COUNT: 734 THERE ARE 734 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 4 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:263579 HCAPLUS
DOCUMENT NUMBER: 133:85417
TITLE: Topography of diphtheria toxin's T domain in the open channel state
AUTHOR(S): Senzel, Lisa; Gordon, Michael; Blaustein, Robert O.; Oh, K. Joon; Collier, R. John; Finkelstein, Alan
CORPORATE SOURCE: Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461, USA
SOURCE: Journal of General Physiology (**2000**), 115(4), 421-434
CODEN: JGPLAD; ISSN: 0022-1295
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB When diphtheria toxin encounters a low **pH** environment, the channel-forming T domain undergoes a poorly understood conformational change that allows for both its own membrane insertion and the translocation of the toxin's catalytic domain across the membrane. From the crystallog. structure of the water-soluble form of diphtheria toxin, a double dagger model was proposed in which 2 transmembrane helical hairpins, TH5-7 and TH8-9, anchor the T domain in the membrane. In this paper, the authors report the topog. of the T domain in the open channel state. This topog. was derived from expts. in which either a hexahistidine (H6) tag or biotin moiety was attached at residues that were

mutated to cysteines. From the sign of the voltage gating induced by the H6 tag and the accessibility of the biotinylated residues to streptavidin added to the cis or trans side of the membrane, the authors determined which segments of the T domain are on the cis or trans side of the membrane and, consequently, which segments span the membrane. There are 3 membrane-spanning segments. Two of them are in the channel-forming piece of the T domain, near its carboxy terminal end, and correspond to one of the proposed daggers, TH8-9. The other membrane-spanning segment roughly corresponds to only TH5 of the TH5-7 dagger, with the rest of that region lying on or near the cis surface. It was also found that, in association with channel formation, the amino terminal third of the T domain, a **hydrophilic** stretch of .apprx.70 residues, is translocated across the membrane to the trans side.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 5 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:809150 HCAPLUS
DOCUMENT NUMBER: 132:325924
TITLE: The effects of nonionic surfactants on the permeation of solutes across Caco-2 cell monolayers
AUTHOR(S): Hugger, Erin D.; Novak, Barbara L.; Borchardt, Ronald T.
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, 66047, USA
SOURCE: Bulletin Technique Gattefosse (1999), 92, 59-65
CODEN: BTGRDQ; ISSN: 0397-7617
PUBLISHER: Gattefosse s.a.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To improve the oral bioavailability of drug candidates, many pharmaceutical companies have synthesized more **hydrophobic** compds. to promote permeation via the transcellular pathway. However, increased **hydrophobicity** causes limited solubility of many drugs. Thus, nonionic surfactants such as Cremophor EL, Tween 80, and PEG are often added to formulations to increase the aqueous solubility of **hydrophobic** drugs. Although some nonionic surfactants inhibit MDR1 (multidrug resistance protein or P-glycoprotein, an apically polarized efflux transporter) activity and membrane fluidity in certain cancer cell lines, surfactant-induced effects on this transporter in the intestinal epithelium have been little examined The effects of nonionic surfactants on the permeation of MDR1 substrates through Caco-2 cell monolayers, an in vitro model of the intestinal epithelium, were studied. The nonionic surfactants Cremophor EL (≤0.1%) and Tween 80 (≤0.05%) increased the apical-to basal effective permeability coefficient (Pe) of Caco-2 cell monolayers for N-acetyl-D-phenylalanyl-N-methyl-D-phenylalanyl-Nα-methyl-D-phenylalaninamide and decreased the basal-to-apical Pe values with increasing surfactant concns., consistent with their altering the activity of MDR1. PEG-300 completely inhibited MDR1 activity in Caco-2 cells by increasing the apical-to-basal transport of taxol, another nonionic surfactant, and decreasing its basal-to-apical transport. PEG-300 at ≤25% did not change the Caco-2 **cell membrane** fluidity when 1,6-diphenyl-1,3,5-hexatriene was used as the fluorescent probe; similar results were obtained with MDCK cells transfected with the gene for MDR1.

L22 ANSWER 6 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:103531 HCAPLUS

DOCUMENT NUMBER: 130:263772
TITLE: Identification of residues lining the translocation pore of human AE1, plasma membrane anion exchange protein
AUTHOR(S): Tang, Xiao-Bo; Kovacs, Miklos; Sterling, Deborah; Casey, Joseph R.
CORPORATE SOURCE: Department of Physiology, University of Alberta, Edmonton, AB, T6G 2H7, Can.
SOURCE: Journal of Biological Chemistry (**1999**), 274(6), 3557-3564
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB AE1 is the chloride/bicarbonate anion exchanger of the erythrocyte plasma membrane. We have used scanning cysteine mutagenesis and sulfhydryl-specific chemical to identify pore-lining residues in the Ser643-Ser690 region of the protein. The Ser643-Ser690 region spans transmembrane segment 8 of AE1 and surrounds Glu681, which may reside at the transmembrane permeability barrier. Glu681 also directly interacts with some anions during anion transport. The introduced cysteine mutants were expressed by transient transfection of HEK293 cells. Anion exchange activity was assessed by measurement of changes of intracellular **pH**, which follow transmembrane bicarbonate movement mediated by AE1. To identify residues that might form part of an aqueous transmembrane pore, we measured anion exchange activity of each introduced cysteine mutant before and after incubation with the sulfhydryl reagents para-chloromercuribenzene sulfonate and 2-(aminoethyl)methanethiosulfonate hydrobromide. Our data identified transmembrane mutants A666C, S667C, L669C, L673C, L677C, and L680C and intracellular mutants I684C and I688C that could be inhibited by sulfhydryl reagents and may therefore form a part of a transmembrane pore. These residues map to one face of a helical wheel plot. The ability to inhibit two intracellular mutants suggests that transmembrane helix 8 extends at least two helical turns beyond the intracellular membrane surface. The identified **hydrophobic** pore-lining residues (leucine, isoleucine, and alanine) may limit interactions with substrate anions.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 7 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:814235 HCAPLUS
DOCUMENT NUMBER: 130:178586
TITLE: Membrane Translocation of Charged Residues at the Tips of **Hydrophobic** Helixes in the T Domain of Diphtheria Toxin
AUTHOR(S): Ren, Jianhua; Sharpe, Juanita C.; Collier, R. John; London, Erwin
CORPORATE SOURCE: Department of Biochemistry and Cell Biology and Department of Chemistry, S.U.N.Y. at Stony Brook, Stony Brook, NY, 11794-5215, USA
SOURCE: Biochemistry (**1999**), 38(3), 976-984
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The low **pH**-triggered membrane insertion of the T domain of diphtheria toxin is a critical step in the translocation of the C domain of the toxin across membranes in vivo. The authors previously established

that the T domain can interact with membranes in two distinct conformations, one in which the TH8/TH9 helical hairpin lies close to the bilayer surface and a second in which it inserts more deeply and appears to be transmembraneous. The loss of charge on residues E349 and D352 due to protonation at low **pH** has been proposed to be a critical step in transmembrane insertion because they are within a loop connecting TH8 and TH9 and must cross the membrane upon transmembrane insertion. In this report, the role of these residues was examined by measuring the effect of the double substitution E349K/D352K on the conformation of the TH8/TH9 hairpin through a fluorescent group attached to TH9. At **pH** 4.5, there was shallower insertion of TH8/TH9 of the E349K/D352K mutant relative to T domain with wild-type residues at 349 and 352. In addition, smaller and(or) fewer pores were obtained with the E349K/D352K mutant relative to the wild-type. On the other hand, high T domain concns., or further decreasing **pH**, allowed transmembrane insertion of both the wild-type and the 349K/352K mutant as well as induction of larger and(or) more numerous pores. Furthermore, the transmembrane insertion process was rapid for both the mutant and wild-type. This shows that the mutant has the capacity to form a transmembrane structure similar to that of the wild-type T domain and, thus, that introduction of charged groups in membrane-penetrating regions of a protein does not introduce an insurmountable barrier to transmembrane movement. The linkage between the ability of the T domain to form the transmembrane conformation and pores suggests that the effects of these mutations in inhibiting pore formation are likely to partly result from the inability to insert properly. Addnl., the observation that decreasing **pH** allows the 349K/352K mutant to insert deeply indicates that there are residues other than E349 and D352 whose protonation promotes transmembrane insertion.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 8 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1998:743887 HCAPLUS
DOCUMENT NUMBER: 130:122600
TITLE: Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs
AUTHOR(S): Urakami, Yumiko; Okuda, Masahiro; Masuda, Satohiro; Saito, Hideyuki; Inui, Ken-Ichi
CORPORATE SOURCE: Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto, 606-8507, Japan
SOURCE: Journal of Pharmacology and Experimental Therapeutics (**1998**), 287(2), 800-805
CODEN: JPETAB; ISSN: 0022-3565
PUBLISHER: Lippencott Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have isolated a kidney-specific organic cation transporter, rat OCT2, which is distinct from rat OCT1 (Okuda M, Saito H, Urakami Y, Takano M and Inui K (1996) Biochem Biophys Res Commun 224:500-507). In our study, the functional characteristics and membrane localization of OCT1 and OCT2 were investigated by uptake studies using MDCK cells transfected with rat OCT1 or OCT2 cDNA (MDCK-OCT1 or MDCK-OCT2) and immunol. studies. Tetraethylammonium (TEA) uptake by both MDCK-OCT1 and MDCK-OCT2 cells was markedly elevated when TEA was added to the basolateral medium, but not to the apical medium. Efflux of TEA from MDCK-OCT1 and MDCK-OCT2 cells was not changed by extracellular **pH** from 5.4 to 8.4, whereas TEA uptake by both transfectants was decreased by acidification of

extracellular medium. Apparent Km values for TEA uptake by MDCK-OCT1 and MDCK-OCT2 cells were 38 and 45 μM, resp. Although various **hydrophilic** organic cations such as 1-methyl-4-phenylpyridinium, cimetidine, quinidine, nicotine, N1-methylnicotinamide and guanidine markedly inhibited TEA uptake by both MDCK-OCT1 and MDCK-OCT2 cells, there were no significant differences in the apparent inhibition consts. (Ki) against these organic cations between both transfectants. Furthermore, immunol. studies using a polyclonal antibody against OCT1 revealed that OCT1 was expressed in the basolateral membranes but not in the brush-border membranes of the rat kidney. These results suggested that both OCT1 and OCT2 are basolateral-type organic cation transporters with broad substrate specificities, mediating tubular secretion of cationic drugs.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 9 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:681004 HCAPLUS
DOCUMENT NUMBER: 130:47449
TITLE: Absorption enhancement, structural changes in tight junctions and cytotoxicity caused by palmitoyl carnitine in Caco-2 and IEC-18 cells
AUTHOR(S): Duizer, Erwin; Van Der Wulp, Cees; Versantvoort, Carolien H. M.; Groten, John P.
CORPORATE SOURCE: Toxicology Division, TNO Nutrition and Food Research Institute, Zeist, Neth.
SOURCE: Journal of Pharmacology and Experimental Therapeutics (**1998**), 287(1), 395-402
CODEN: JPETAB; ISSN: 0022-3565
PUBLISHER: Williams & Wilkins
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Palmitoyl carnitine chloride (PCC) has been shown to be an effective enhancer of intestinal transport of **hydrophilic** mols. The exact mechanism by which the epithelial barrier function is decreased is not clear. To elucidate the mechanism of action of PCC, we studied the relation among absorption enhancement, cell viability and tight junction protein localization in the human colonic Caco-2 cell line and the rat small intestinal cell line IEC-18. Filter-grown cells were exposed to 0 to 1 mM PCC for 30 min, and the efficacy of PCC treatment was determined by assessing the transepithelial elec. resistance and the apparent permeability for mannitol and PEG-4000. Membrane lysis and cytotoxicity were assessed by measurement of lactate dehydrogenase leakage and uptake of propidium iodide and neutral red. The immunolocalization of the tight junctional protein ZO-1 was quantified using CSLM and image-processing software. In both cell lines, PCC caused a dose-dependent decrease in transepithelial elec. resistance and a concomitant increase in the permeability for mannitol and PEG-4000. The transport enhancement was accompanied by an increase in apical membrane permeability and a reduction in cell viability. At higher PCC concns. (≥0.4 mM), the distribution of the tight junctional protein ZO-1 was changed and cells were unable to recover viability. PCC is effective as an absorption enhancer for **hydrophilic** macromols. However, lytic effects on the **cell membrane** and reduced cell viability were concomitant with transport enhancement.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 10 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:312410 HCAPLUS

DOCUMENT NUMBER: 129:78144
TITLE: The Na+-phosphate cotransport system (NaPi-II) with a cleaved protein backbone: implications on function and membrane insertion
AUTHOR(S): Kohl, Beate; Wagner, Carsten A.; Huelseweh, Birgit; Busch, Andreas E.; Werner, Andreas
CORPORATE SOURCE: Max-Planck-Institut fur molekulare Physiologie, Abteilung Epithelphysiologie, Dortmund, 44139, Germany
SOURCE: Journal of Physiology (Cambridge, United Kingdom) (**1998**), 508(2), 341-350
CODEN: JPHYA7; ISSN: 0022-3751
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Renal handling of inorg. phosphate (Pi) involves a Na+-Pi cotransport system, which is well conserved between vertebrates. The members of this protein family, denoted NaPi-II, share a topol. with, it is thought, eight transmembrane domains. The transporter is proposed to be proteolytically cleaved within a large **hydrophilic** loop in vivo. The consequences of an interrupted backbone were tested by constructing cDNA clones encoding different N- (1-3 and 1-5) and C-terminal (4-8 and 6-8) complementary fragments of NaPi-II from winter flounder. When the cognate fragments were used in combination (1-3 plus 4-8; 1-5 plus 6-8), they comprised the full complement of the putative transporter domains. None of the four individual fragments or the 1-5 plus 6-8 combination when expressed in Xenopus oocytes increased Pi flux. Coexpression of fragments 1-3 plus 4-8 stimulated transport activity identical to that for expressed wild-type NaPi-II with regard to **pH** dependency and Km for Na+ and Pi binding; however, the maximal transport rate (vmax) was lower. Immunohistochem. on cryosections confined the functionally active 1-3 plus 4-8 combination to the oocyte membrane. This was not the case for the 1-5 plus 6-8 combination or any of the individual fragments, all of which failed to induce fluorescence. A second immunohistochem. approach using intact oocytes allowed determination of the extracellular regions of the protein.

Epitopes within the loop between transmembrane domains 3 and 4 enhanced fluorescence. Neither N- nor C-terminal tags induced fluorescence.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 11 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:230856 HCAPLUS
DOCUMENT NUMBER: 129:32207
TITLE: Immobilized liposome and biomembrane partitioning chromatography of drugs for prediction of drug transport
AUTHOR(S): Beigi, Farideh; Gottschalk, Ingo; Lagerquist Hagglund, Christine; Haneskog, Lars; Brekkan, Eggert; Zhang, Yanxiao; Osterberg, Thomas; Lundahl, Per
CORPORATE SOURCE: Biomedical Center, Department of Biochemistry, Uppsala University, Uppsala, S-751 23, Swed.
SOURCE: International Journal of Pharmaceutics (**1998**), 164(1-2), 129-137
CODEN: IJPHDE; ISSN: 0378-5173
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Drug partitioning into lipid bilayers was studied by chromatog. on liposomes and biomembranes immobilized in gel beads by freeze-thawing. The drug retention volume was expressed as a capacity factor, Ks, normalized

with respect to the amount of immobilized phospholipid. Log Ks values for pos. charged drugs on brain phosphatidylserine (PS)/egg phosphatidylcholine (PC) liposomes decreased as the ionic strength was increased, increased as the PS:PC ratio or the **pH** was increased and varied linearly with the temperature Log Ks values for beta-blockers, phenothiazines and benzodiazepines on egg phospholipid (EPL) liposomes correlated well with corresponding values on red **cell membrane** lipid liposomes (r2=0.96), and on human red **cell membrane** vesicles containing transmembrane proteins (r2=0.96). A fair correlation was observed between the values on EPL liposomes and those on native membranes of adsorbed red cells (r2=0.86). Compared to the data obtained with liposomes, the retentions of **hydrophilic** drugs became larger and the range of log Ks values more narrow on the vesicles and the membranes, which expose **hydrophilic** protein surfaces and oligosaccharides. Lower correlations were observed between drug retention on EPL liposomes and egg PC liposomes; and between retention on liposomes (or vesicles) and immobilized artificial membrane (IAM) monolayers of PC analogs. Absorption of orally administered drugs in humans (literature data) was nearly complete for drugs of log Ks values in the interval 1.2-2.5 on vesicles. Both vesicles and liposomes can thus be used for chromatog. anal. of drug-membrane interaction and prediction of drug absorption.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 12 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:226537 HCAPLUS
DOCUMENT NUMBER: 129:1739
TITLE: Fatty acid transport: difficult or easy?
AUTHOR(S): Hamilton, James A.
CORPORATE SOURCE: Department of Biophysics, Center for Advanced Biomedical Research, Boston University School of Medicine, Boston, MA, 02118, USA
SOURCE: Journal of Lipid Research (**1998**), 39(3), 467-481
CODEN: JLPRAW; ISSN: 0022-2275
PUBLISHER: Lipid Research, Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 67 refs. Transport of unesterified fatty acids (FA) into cells has been viewed either as a simple diffusion process regulated mainly by lipid phys. chemical or as a more complex process involving protein catalysis. In this review FA transport in cell membranes is broken down into three essential steps: adsorption, transmembrane movement, and desorption. The phys. properties of FA in aqueous, membrane, and protein environments relevant to transport mechanisms are discussed, with emphasis on recent information derived from NMR and fluorescence studies. Because of their low solubility in water and high **hydrophobicity**, FA bind rapidly and avidly to model membranes (phospholipid bilayers); if albumin is a donor, FA desorb rapidly to reach their equilibrium distribution between the membrane and albumin. The ionization properties of FA in a phospholipid bilayer result in a high population of the un-ionized form (.apprx.50%) at **pH** 7.4, which diffuses across the lipid bilayer (flip-flops) rapidly (t1/2 < 1 s). Desorption of FA from a phospholipid surface is slower than transmembrane movement and dependent on the FA chain length and unsatn., but is rapid for typical dietary FA. These phys. properties of FA in model systems predict that proteins are not essential for transport of FA through membranes. The only putative FA transport protein to be purified and reconstituted into phospholipid bilayers, the mitochondrial uncoupling protein (UCP1), was shown to

transport the FA anion in response to FA flip-flop. New expts. with cells have found that FA movement into cells acidifies the cytosol, as predicted by the flip-flop model.

REFERENCE COUNT: 67 THERE ARE 67 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 13 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:358076 HCAPLUS
DOCUMENT NUMBER: 127:91919
TITLE: Calcium- and **pH**-linked oligomerization of sorcin causing translocation from cytosol to membranes
AUTHOR(S): Zamparelli, Carlotta; Ilari, Andrea; Verzili, Daniela; Vecchini, Paola; Chiancone, Emilia
CORPORATE SOURCE: CNR Center of Molecular Biology, Department of Biochemical Sciences A. Rossi Fanelli', Universita La Sapienza, Rome, 00185, Italy
SOURCE: FEBS Letters (**1997**), 409(1), 1-6
CODEN: FEBLAL; ISSN: 0014-5793
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sorcin, a cytosolic calcium-binding protein containing a pair of EF-hand motifs, undergoes a Ca2+-dependent translocation to the **cell membrane.** The underlying conformational change is similar at **pH** 6.0 and 7.5 and consists in an increase in overall **hydrophobicity** that involves the aromatic residues and in particular the two tryptophan residues which become less exposed to solvent. The concomitant association from dimers to tetramers indicates that the tryptophan residues, which are located between the EF-hand sites, become buried at the dimer-dimer interface. Ca2+-bound sorcin displays a striking difference in solubility as a function of **pH** that has been ascribed to the formation of calcium-stabilized aggregates.

L22 ANSWER 14 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1996:725586 HCAPLUS
DOCUMENT NUMBER: 126:14338
TITLE: Correlation of trimethoprim and brodimoprim physicochemical and lipid membrane interaction properties with their accumulation in human neutrophils
AUTHOR(S): Fresta, Massimo; Furneri, Pio M.; Mezzasalma, Elena; Nicolosi, Vito M.; Puglisi, Giovanni
CORPORATE SOURCE: Dep. Pharmaceutical Sci., Univ. Catania, Catania, I-95125, Italy
SOURCE: Antimicrobial Agents and Chemotherapy (**1996**), 40(12), 2865-2873
CODEN: AMACCQ; ISSN: 0066-4804
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Dipalmitoylphosphatidylcholine vesicles were used as a biol. membrane model to investigate the interaction and the permeation properties of trimethoprim and brodimoprim as a function of drug protonation. The drug-membrane interaction was studied by differential scanning calorimetry. Both drugs interacted with the **hydrophilic** phospholipid head groups when in a protonated form. An experiment on the permeation of the two drugs through dipalmitoylphosphatidylcholine biomembranes showed higher diffusion rate consts. when the two drugs were in the uncharged form; lowering of the **pH** (formation of protonated species) caused a reduction of permeation. Drug uptake by human

neutrophil cells was also investigated. Both drugs may accumulate within neutrophils; however, brodimoprim does so to a greater extent. This accumulation is probably due to a **pH** gradient driving force, which allows the two drugs to move easily from the extracellular medium (**pH**.apprx.7.3) into the internal cell compartments (acid **pH**). Once protonated, both drugs are less able to permeate and can be trapped by the neutrophils. This investigation showed the importance of the physicochem. properties of brodimoprim and trimethoprimin determining drug accumulation and membrane permeation pathways.

L22 ANSWER 15 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1996:692614 HCAPLUS
DOCUMENT NUMBER: 126:45323
TITLE: Temperature dependence of the exchange of monovalent anions in human red blood cells
AUTHOR(S): Baker, Graham F.; Baker, Petroulla
CORPORATE SOURCE: Department of Biological Sciences, Royal Holloway, University of London, Egham Hill, Egham Surrey, TW20 0EX, UK
SOURCE: Biochimica et Biophysica Acta, Biomembranes (**1996**), 1285(2), 192-202
CODEN: BBBMBS; ISSN: 0005-2736
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The temperature dependence of anion exchange across the red **cell membrane** was studied between 5°C and 55°C by measuring the rate of shrinkage of cells when transferred from a medium of **pH** 7.6 to one of **pH** 9.3 (as measured at 22°C). The rates of shrinkage varied with the anion studied, the order being F->Cl->Br->I->SCN- but were faster in the presence of trace amts. of carbon dioxide than in its absence. NO3- was as fast as Cl- when carbon dioxide was present but comparable with I- when it was removed. Arrhenius plots of the rates were linear for all anions over the temperature range studied and gave the following apparent activation energies in kJ·mol-1; F-, 67.7; NO3-, 68.4; Cl-, 70.2; Br-, 79.6; SCN-, 87.4 and I-, 95.1 in the presence of carbon dioxide. Inhibition of carbonic anhydrase with 5 μm ethoxzolamide and the removal of the carbon dioxide by degassing raised the activation energies to; F-, 124.8; NO3-, 129.0; Cl-, 141.5; Br-, 159.4; SCN-, 150.0 and I-, 185.6 kJ·mol-1. With the exception of F-, the apparent activation energies of the anions were linearly related to their thermochem. (dehydrated) radii in both cases. The relationship between the ionic radii and the energy of transfer suggests that anion exchange involves transfer through a **hydrophobic** pathway and that addnl. energy is required to overcome restrictions experienced in passing through the pathway. It is proposed that this, rather than a conformational change in the protein dets. the activation energy of the process.

L22 ANSWER 16 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1996:511874 HCAPLUS
DOCUMENT NUMBER: 125:184568
TITLE: Membrane energization by proton pumps is important for compartmentalization of drugs and toxins: a new type of active transport
AUTHOR(S): Moriyama, Yoshinori
CORPORATE SOURCE: Graduate School of Gene Sciences, Hiroshima University, Hiroshima, 722, Japan
SOURCE: Journal of Experimental Biology (**1996**),

199(7), 1447-1454
CODEN: JEBIAM; ISSN: 0022-0949
PUBLISHER: Company of Biologists
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review, with refs. Many organelles are energized by proton pumps: mitochondria form an inside-neg. membrane potential by means of the respiratory chain and endomembrane structures, such as lysosomes and synaptic vesicles, establish an internal acidic **pH** by means of a vacuolar-type H+-ATPase (V-ATPase). Various amphipathic drugs such as local anesthetics and neuron blockers are accumulated in acidic organelles upon energization by proton pumps. However, this process does not require any transporters specific for the drugs: these drugs penetrate through the lipid bilayer against a concentration gradient to accumulate inside the energized organelles. Essentially the same transport process takes place in liposomes that have been reconstituted with purified V- or F-ATPase. Various **hydrophobic** cations are also accumulated in mitochondria by a similar mechanism. The energy-dependent but transporter-independent accumulation does not belong to the known transport categories and seems to represent a new type of transport which may be important for understanding the mode of action of drugs and toxins.

L22 ANSWER 17 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1996:434961 HCAPLUS
DOCUMENT NUMBER: 125:76328
TITLE: Active agent transport systems using perturbants to convert active agent to state between native and denatured states
INVENTOR(S): Milstein, Sam J.; Barantsevitch, Evgueni; Leone-Bay, Andrea; Wang, Nai Fang; Sarubbi, Donald J.; Santiago, Noemi B.
PATENT ASSIGNEE(S): Emisphere Technologies, Inc., USA
SOURCE: PCT Int. Appl., 119 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 30
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9612475	A1	19960502	WO 1995-US14598	19951024 <--
W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5714167	A	19980203	US 1994-328932	19941025 <--
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AU 9641524	A1	19960515	AU 1996-41524	19951024 <--
EP 781124	A1	19970702	EP 1995-939863	19951024 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 10509433	T2	19980914	JP 1996-514159	19951024 <--
AU 771024	B2	20040311	AU 2000-72261	20001214 <--
AU 771434	B2	20040325	AU 2000-72260	20001214 <--
AU 2004202745	A1	20040923	AU 2004-202745	20040623
PRIORITY APPLN. INFO.:			US 1994-328932 A	19941025 <--

US 1992-898909	B2	19920615 <--
US 1992-920346	A2	19920727 <--
US 1993-51019	YY	19930422 <--
US 1993-76803	A2	19930614 <--
US 1993-143571	B2	19931026 <--
US 1993-168776	A2	19931216 <--
US 1994-205511	A2	19940302 <--
US 1994-231622	A2	19940422 <--
US 1994-231623	A2	19940422 <--
US 1994-315200	A2	19940929 <--
US 1994-316404	A2	19940930 <--
WO 1995-US14598	W	19951024 <--
AU 1998-62756	A3	19980206 <--
AU 2000-72260	A3	20001214

OTHER SOURCE(S): MARPAT 125:76328

AB Methods are disclosed for transporting a biol. active agent across a cellular membrane or a lipid bilayer. A first method includes the steps of: (a) providing a biol. active agent which can exist in a native conformational state, a denatured conformational state, and an intermediate conformational state which is reversible to the native state and which is conformationally between the native and denatured states; (b) exposing the biol. active agent to a complexing perturbant to reversibly transform the biol. active agent to the intermediate state and to form a transportable supramol. complex; and (c) exposing the membrane or bilayer to the supramol. complex, to transport the biol. active agent across the membrane or bilayer. The perturbant has a mol. weight between about 150 and about 600 daltons, and contains at least one **hydrophilic** moiety and at least one **hydrophobic** moiety. The supramol. complex comprises the perturbant noncovalently bound or complexed with the biol. active agent. In the present invention, the biol. active agent does not form a microsphere after interacting with the perturbant. A method for preparing an orally administrable biol. active agent comprising steps (a) and (b) above is also provided as are oral delivery compns. Addnl., mimetics and methods for preparing mimetics are contemplated. The methods and compns. of the invention facilitate the delivery of an active agent to a target, e.g. the delivery of a pharmaceutical through an adverse environment to a particular location in the body. The biol. active agent may be e.g. a carbohydrate, mucopolysaccharide, lipid, pesticide, or peptide, e.g. human or bovine growth hormone, an interferon, insulin, an antigen, a monoclonal antibody, cromolyn sodium, vancomycin, heparin, etc. The perturbant may be e.g. a proteinoid, carboxylic acid, or acylated amino acid or poly(amino acid). The perturbant may also be a **pH**-changing agent, an ionic strength-changing agent, or guanidine-HCl.

L22 ANSWER 18 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1996:210308 HCAPLUS

DOCUMENT NUMBER: 124:253099

TITLE: Roles of Glu 349 and Asp 352 in membrane insertion and translocation by diphtheria toxin

AUTHOR(S): Kaul, Poonam; Silverman, Jared; Shen, Wei Hai; Blanke, Steven R.; Huynh, Paul D.; Finkelstein, Alan; Collier, R. John

CORPORATE SOURCE: Dep. Microbiology Molecular Genetics, Harvard Medical School, Boston, MA, 02115, USA

SOURCE: Protein Science (**1996**), 5(4), 687-92
CODEN: PRCIEI; ISSN: 0961-8368

PUBLISHER: Cambridge University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Acidic conditions within the **endosomal** lumen induce the T domain

of receptor-bound diphtheria toxin (DT) to insert into the **endosomal** membrane and mediate translocation of the toxin's catalytic domain to the cytosol. A conformational rearrangement in the toxin occurring near **pH** 5 allows a buried apolar helical hairpin of the native T domain (helixes TH8 and TH9) to undergo membrane insertion. If the inserted hairpin spans the bilayer, as hypothesized, then the two acidic residues within the TL5 interhelical loop, Glu 349 and Asp 352, should become exposed at the neutral cytosolic face of the membrane and re-ionize. To investigate the roles of these residues in toxin action, the authors characterized mutant toxins in which one or both acidic residues had been replaced with non-ionizable ones. Each of two double mutants examined showed a several-fold reduction in cytotoxicity in 24-h Vero cell assays (sixfold for E349A+D352A and fourfold for E349Q+D352N), whereas the individual E349Q and D352N mutations caused smaller redns. in toxicity. The single and double mutations also attenuated the toxin's ability to permeabilize Vero cells to Rb+ at low **pH** and decreased channel formation by the toxin in artificial planar bilayers. Neither of the double mutations affected the **pH**-dependence profile of the toxin's conformational rearrangement in solution, as measured by binding of the **hydrophobic** fluorophore, 2-p-toluidinyl-naphthalene 6-sulfonate. The results demonstrate that, although there is no absolute requirement for an acidic residue within the TL5 loop for toxicity, Glu 349 and Asp 352 do significantly enhance the biol. activity of the protein. The data are consistent with a model in which ionization of these residues at the cytosolic face of the **endosomal** membrane stabilizes the TH8/TH9 hairpin in a transmembrane configuration, thereby facilitating channel formation and translocation of the toxin's catalytic chain.

L22 ANSWER 19 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1995:998470 HCAPLUS
DOCUMENT NUMBER: 124:75936
TITLE: Mechanism of K+ channel block by verapamil and related compounds in rat alveolar epithelial cells
AUTHOR(S): DeCoursey, Thomas E.
CORPORATE SOURCE: Department of Molecular Biophysics and Physiology, Rush Presbyterian St. Luke's Medical Center, Chicago, IL, 60612, USA
SOURCE: Journal of General Physiology (**1995**), 106(4), 745-79
CODEN: JGPLAD; ISSN: 0022-1295
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The mechanism by which the phenylalkylamines, verapamil and D600, and related compds., block inactivating delayed rectifier K+ currents in rat alveolar epithelial cells, was investigated using whole-cell tight-seal recording. Block by phenylalkylamines added to the bath resembles state-dependent block of squid K+ channels by internally applied quaternary ammonium ions (Armstrong, C. M. 1971): open channels are blocked preferentially, increased [K+]o accelerates recovery from block, and recovery occurs mainly through the open state. Slow recovery from block is attributed to the existence of a blocked-inactivated state, because recovery was faster in three situations where recovery from inactivation is faster: (a) at high [K+]o, (b) at more neg. potentials, and (c) in cells with type 1 K+ channels, which recover rapidly from inactivation. The block rate was used as a bioassay to reveal the effective concentration of drug at the block site. When external **pH**, pHo, was varied, block was much faster at pHo 10 than pHo 7.4, and very slow at pHo 4.5. The block rate was directly proportional to the concentration

of neutral drug in the bath, suggesting that externally applied drug must enter the membrane in neutral form to reach the block site. High internal **pH** (pHi 10) reduced the apparent potency of externally applied phenylalkylamines, suggesting that the cationic form of these drugs blocks K+ channels at an internal site. The permanently charged analog D890 blocked more potently when added to the pipet than to the bath. However, lowering pHi to 5.5 did not enhance block by external drug, and tertiary phenylalkylamines added to the pipet solution blocked weakly. This result can be explained if drug diffuses out of the cell faster than it is delivered from the pipet, the block site is reached preferentially via **hydrophobic** pathways, or both. Together, the data indicate the neutral membrane-bound drug blocks K+ channels more potently than intracellular cationic drug. Neutral drug has rapid access to the receptor, where block is stabilized by protonation of the drug from the internal solution In summary, externally applied phenylalkylamines block open or inactivated K+ channels by partitioning into the **cell membrane** in neutral form and are stabilized at the block site by protonation.

L22 ANSWER 20 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1994:675163 HCAPLUS
DOCUMENT NUMBER: 121:275163
TITLE: Activation of the Dunaliella acidophila plasma membrane H+-ATPase by trypsin cleavage of a fragment that contains a phosphorylation site
AUTHOR(S): Sekler, Israel; Weiss, Meira; Pick, Uri
CORPORATE SOURCE: Dep. Biochem., Weizmann Inst. Sci., Rehovot, 76100, Israel
SOURCE: Plant Physiology (**1994**), 105(4), 1125-32
CODEN: PLPHAY; ISSN: 0032-0889
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Trypsin treatment of purified H+-ATPase from plasma membranes of the extreme acidophilic alga Dunaliella acidophila enhances ATP hydrolysis and H+ pumping activities. The activation is associated with an alkaline **pH** shift, an increase in Vmax, and a decrease in Km(ATP). The activation is correlated with cleavage of the 100 kDa ATPase polypeptide to a fragment of approx. 85 kDa and the appearance of three minor **hydrophobic** fragments of 7 to 8 kDa, which remain associated with the major 85 kDa polypeptide. The N-terminal sequence of the small fragments has partial homol. to residues 713 to 741 of Arabidopsis thaliana plasma membrane H+-ATPase. Incubation of cells with 32P-labeled orthophosphate (32Pi) results in incorporation of 32P into the ATPase 100 kDa polypeptide. Trypsin treatment of the 32Pi-labeled ATPase leads to complete elimination of label from the approx. 85 kDa polypeptide. Cleavage of the phosphorylated enzyme with endoproteinase Glu-C (V-8) yields a phosphorylated 12 kDa fragment. Peptide mapping comparison between the 100 kDa and the trypsinized 85 kDa polypeptides shows that the 12 kDa fragment is derived from the trypsin-cleaved part of the enzyme. The N-terminal sequence of the 12 kDa fragment closely resembles a C-terminal stretch of an ATPase from another Dunaliella species. It is suggested that trypsin activation of the D. acidophila plasma membrane H+-ATPase results from elimination of an autoinhibitory domain at the C-terminal end of the enzyme that carries a vicinal phosphorylation site.

L22 ANSWER 21 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1994:526778 HCAPLUS
DOCUMENT NUMBER: 121:126778
TITLE: Quinolone resistance mediated by norA: physiologic characterization and relationship to flqB, a quinolone

resistance locus on the Staphylococcus aureus chromosome
AUTHOR(S): Ng, Eva Y. W.; Trucksis, Michele; Hooper, David C.
CORPORATE SOURCE: Infect. Dis. Unit, Massachusetts Gen. Hosp., Boston, MA, 02114-2696, USA
SOURCE: Antimicrobial Agents and Chemotherapy (**1994**), 38(6), 1345-55
CODEN: AMACCQ; ISSN: 0066-4804
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors identified a quinolone resistance locus, flqB, linked to transposon insertion Ω1108 and fus on the SmaI D fragment of the Staphylococcus aureus NCTC 8325 chromosome, the same fragment that contains the norA gene. S. aureus norA cloned from flqB and flqB+ strains in Escherichia coli differed only in a single nucleotide in the putative promoter region. There was no detectable change in the number of copies of norA on the chromosomes of flqB strains, but they had increased levels of norA transcripts. Cloned norA produced resistance to norfloxacin and other **hydrophilic** quinolones and reduced norfloxacin accumulation in intact cells that was energy dependent, suggesting active drug efflux as the mechanism of resistance. Drug efflux was studied by measurement of norfloxacin uptake into everted inner membrane vesicles prepared from norA-containing E. coli cells. Vesicles exhibited norfloxacin uptake after the addition of lactate or NADH, and this uptake was abolished by carbonyl cyanide m-chlorophenylhydrazone and nigericin but not valinomycin, indicated that it was linked to the **pH** gradient across the **cell membrane.** Norfloxacin uptake into vesicles was also saturable, with an apparent Km of 6 μM, a concentration between those that inhibit the growth of flqB and flqB+ S. aureus cells, indicating that drug uptake is mediated by a carrier with a high apparent affinity for norfloxacin. Ciprofloxacin and ofloxacin competitively inhibited norfloxacin uptake into vesicles. Reserpine, which inhibits the multidrug efflux mediated by the bmr gene of Bacillus subtilis, which is similar to norA, abolished norfloxacin uptake into vesicles as well as the norfloxacin resistance of an flqB mutant, suggesting a potential means for circumventing quinolone resistance as a result of drug efflux in S. aureus. These findings indicate that the chromosomal flqB resistance locus is associated with increased levels of expression of norA and strongly suggest that the NorA protein itself functions as a drug transporter that is coupled to the proton gradient across the **cell membrane.**

L22 ANSWER 22 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1994:74203 HCAPLUS
DOCUMENT NUMBER: 120:74203
TITLE: The effects of PMA and TFP and alterations in intracellular **pH** and calcium concentration on the membrane associations of phospholipid-binding proteins fodrin, protein kinase C and annexin II in cultured MDCK cells
AUTHOR(S): Vaeaeraeniemi, Jukka; Huotari, Virva; Lehto, Veli-Pekka; Eskelinen, Sinikka
CORPORATE SOURCE: Biocenter and Department of Pathology, University of Oulu, Kajaanintie 52 D, Oulu, FIN-90220, Finland
SOURCE: Biochimica et Biophysica Acta, Biomembranes (**1994**), 1189(1), 21-30
CODEN: BBBMBS; ISSN: 0005-2736
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Annexin II, α-fodrin and protein kinase C (PKC) are associated with the cytoplasmic surface of the plasma membranes. When assayed with liposomes, they show affinity for acidic phospholipids and bind Ca2+. They also respond to or participate in cell signal transduction by altered membrane binding properties. In the present work the authors have studied the properties of these proteins in epithelial MDCK cells in response to elevated intracellular Ca2+ concentration, lowered **pH**, treatment with tumor promoter phorbol myristoyl acetate (PMA) and calmodulin inhibitor trifluoperazine (TFP). In untreated polarized MDCK cells annexin II was seen both along the lateral walls and membranes of intracellular vesicles, fodrin was located along the lateral walls, whereas PKC was seen in the cytoplasm. There was no observable translocation of these proteins upon elevation of the intracellular calcium concentration using a calcium ionophore A 23187. Treatment with TFP led to a release of annexin II from the plasma membranes which was accompanied by a transient peak in the intracellular calcium. Treatment with PMA led to a loss of the cubic form of the cells, a slight elevation in the intracellular calcium concentration, and a drop in the intracellular **pH**. Simultaneously fodrin was released from the lateral walls, but still remained insol. in Triton X-100, PKC became associated with the intracellular membranes and fibers, whereas annexin II remained along the lateral walls. These changes could be prevented by clamping the intracellular **pH** neutral during PMA treatment. Lowering of intracellular **pH** below 6.5 with the nigericin treatment led to a similar translocation of fodrin and PKC as PMA. This suggests that the protein redistribution is caused by cytoplasmic acidification and is due to an increased **hydrophobicity** and enhanced protonation of lipids and proteins. In contrast, no changes were seen in the annexin II distribution in response to altered **pH**. Hence, its release by TFP is presumably due to changes in the cationic properties of the inner phase of the plasma membrane. Thus, proteins which show similar binding properties with liposomes show different characteristics in their association with the intracellular membranes.

L22 ANSWER 23 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:618554 HCAPLUS
DOCUMENT NUMBER: 119:218554
TITLE: Expression cloning in yeast of a cDNA encoding a broad specificity amino acid permease from Arabidopsis thaliana
AUTHOR(S): Frommer, Wolf B.; Hummel, Sabine; Riesmeier, Joerg W.
CORPORATE SOURCE: Abt. Willmitzer, Inst. Genbiol. Forsch., Berlin, D-1000/33, Germany
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (**1993**), 90(13), 5944-8
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To study amino acid transport in plants at the mol. level, the authors isolated an amino acid permease cDNA from Arabidopsis thaliana by complementation of a yeast mutant defective in proline uptake with a cDNA. The predicted polypeptide of 53 kDa is highly **hydrophobic** with 12 putative membrane-spanning regions and shows no significant homologies to other known transporters. Expression of the cDNA enables the yeast mutant to take up L-[14C]proline. Competition studies argue for a broad but stereospecific substrate recognition by the permease, which resembles neutral or general amino acid transport systems from Chlorella and higher plants. Both **pH** dependence and inhibition by protonophores are

consistent with a proton symport mechanism.

L22 ANSWER 24 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:250610 HCAPLUS
DOCUMENT NUMBER: 118:250610
TITLE: Mutagenesis of conserved residues in the phosphorylation domain of the yeast plasma membrane hydrogen ion-ATPase. Effects on structure and function
AUTHOR(S): Rao, Rajini; Slayman, Carolyn W.
CORPORATE SOURCE: Sch. Med., Yale Univ., New Haven, CT, 06510, USA
SOURCE: Journal of Biological Chemistry (1993), 268(9), 6708-13
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A **diagnostic** feature of P-ATPases is a phosphorylation motif (DKTGTLT), located in the **hydrophilic** center of the polypeptide chain, within which the β-aspartyl-phosphate reaction intermediate is formed. The roles of four invariant residues (Lys379, Thr380, Thr382, and Thr384) in this region of the yeast plasma membrane H+-ATPase have been analyzed by site-directed mutagenesis. In addition, a set of six insertion mutants was generated containing a single glycine residue at each of the indicated sites: CVSVDVKVTVGTVLT to examine spatial arrangements within this highly conserved domain. In order to minimize toxic effects of the mutations on cell growth, the defective ATPases were expressed behind an inducible heat shock promoter and targeted to an intracellular pool of secretory vesicles, while wild-type ATPase was maintained in the plasma membrane where it is required for viability. Secretory vesicles containing mutant ATPase were isolated as described previously and assayed for the amount of ATPase polypeptide and for rates of ATP hydrolysis and H+ pumping. All of the insertion mutations led to biosynthetic arrest of the defective enzyme, with no ATPase appearing in the secretory vesicles. Nonconservative amino acid substitutions (Lys→Gln, Thr→Ala) inactivated the ATPase, whereas conservative substitutions (Lys→Arg, Thr→Ser) retained partial activity which has been characterized in detail. There was little or no change in the Km for ATP or the **pH** optimum in any of the mutant enzymes. Strikingly, however, all displayed an increase in resistance to vanadate, consistent with the idea that the residues in question contribute to a phosphate/vanadate binding site or that they affect the equilibrium between E1 and E2 conformations of the enzyme.

L22 ANSWER 25 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:186541 HCAPLUS
DOCUMENT NUMBER: 118:186541
TITLE: Purification and properties of a plasma membrane hydrogen ion-ATPase from the extremely acidophilic alga Dunaliella acidophila
AUTHOR(S): Sekler, Israel; Pick, Uri
CORPORATE SOURCE: Dep. Biochem., Weizmann Inst. Sci., Rehovot, 76100, Israel
SOURCE: Plant Physiology (1993), 101(3), 1055-61
CODEN: PLPHAY; ISSN: 0032-0889
DOCUMENT TYPE: Journal
LANGUAGE: English
AB This paper describes partial purification and characterization of vanadate-sensitive H+-ATPase from plasma membranes of D. acidophila, an extremely acidophilic unicellular alga. Purification is based on the insoly. in and stability of the enzyme in Triton X-100. The purified enzyme is highly enriched in a polypeptide of mol. mass 100 kD, which cross-reacts

with antibodies against the plant plasma membrane H+-ATPase. Upon reconstitution into proteoliposomes, the enzyme catalyzes an ATP-dependent electrogenic H+ uptake. ATP hydrolysis is stimulated by lipids, is inhibited by vanadate, diethylstilberstrol, dicyclohexylcarbodimide, erythrosine, and mercurials, and shows a sharp optimum at **pH** 6. Unusual properties of this enzyme, by comparison with plant plasma membrane H+-ATPases, are a higher affinity for ATP (Km = 40 μM) and larger stimulation by K+, which interacts with the enzyme from its cytoplasmic side. Comparative studies with cross-reacting antibodies, prepared against different domains of the plant H+-ATPase, suggest that the central **hydrophilic** domain containing the catalytic site is more conserved than the C- and N-terminal ends. The high abundance and stability of the plasma membrane H+-ATPase from D. acidophila make it an attractive model system for studies of the structure-function relations and regulation of this crucial enzyme.

L22 ANSWER 26 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1992:525517 HCAPLUS
DOCUMENT NUMBER: 117:125517
TITLE: Coexistence of the genes for putrescine transport protein and ornithine decarboxylase at 16 min on Escherichia coli chromosome
AUTHOR(S): Kashiwagi, Keiko; Suzuki, Tomoko; Suzuki, Fumihiro; Furuchi, Takemitsu; Kobayashi, Hiroshi; Igarashi, Kazuei
CORPORATE SOURCE: Fac. Pharm. Sci., Chiba Univ., Chiba, 260, Japan
SOURCE: Journal of Biological Chemistry (**1991**), 266(31), 20922-7
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nucleotide sequence of one of the putrescine transport operons (pPT71), located at 16 min of the E. coli chromosome, was determined It contained the genes for an induced ornithine decarboxylase and a putrescine transport protein. The gene for the ornithine decarboxylase contained a 2196-nucleotide open reading frame encoding a 732-amino acid protein whose calculated Mr was 82,414 and the predicted amino acid sequence from the open reading frame had 65% homol. with that of a constitutive ornithine dearboxylase encoded by the gene at 64 min. The ornithine decarboxylase activity was observed in the cells carrying pPT71 cultured at **pH** 5.2, but not in the cells cultured at **pH** 7.0. The gene for the putrescine transport protein contained a 1317-nucleotide open reading frame encoding a 439-amino acid protein whose calculated Mr was 46,494. The hydropathy profile of the putrescine transport protein revealed that it consisted of 12 putative transmembrane scanning segments linked by **hydrophilic** segments of variable length. The transport protein was in fact found in the membrane fraction. When the gene for the putrescine transport protein was linked to the test promoter of the vector instead of its own promoter, the putrescine transport activity increased greatly. The results suggest that the gene expression of the operon is repressed strongly under standard conditions.

L22 ANSWER 27 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1991:402889 HCAPLUS
DOCUMENT NUMBER: 115:2889
TITLE: Peptides fused to the amino-terminal end of diphtheria toxin are translocated to the cytosol
AUTHOR(S): Stenmark, Harald; Moskaug, Jan Oeivind; Madshus, Inger Helene; Sandvig, Kirsten; Olsnes, Sjur
CORPORATE SOURCE: Inst. Cancer Res., Norw. Radium Hosp., Oslo, N-0310,

Norway
SOURCE: Journal of Cell Biology (**1991**), 113(5), 1025-32
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of NH2-terminal extensions of diphtheria toxin on its ability to become translocated to the cytosol was investigated. DNA fragments encoding peptides of 12-30 amino acids were fused by recombinant DNA technol. to the 5'-end of the gene for a mutant toxin. The resulting DNA constructs were transcribed and translated in vitro. The translation products were bound to cells and then exposed to low **pH** to induce translocation across the **cell membrane.** Under these conditions all of the oligopeptides tested, including 3 viral peptides and the leader peptide of diphtheria toxin, were translocated to the cytosol along with the enzymic part (A-fragment) of the toxin. Neither **hydrophobic** nor highly charged sequences blocked translocation. The results are compatible with a model in which the COOH-terminus of the A-fragment 1st crosses the membrane, whereas the NH2-terminal region follows behind. The possibility of using nontoxic variants of diphtheria toxin as vectors to introduce peptides into the cytosol to elicit MHC class I-restricted immune response and clonal expansion of the relevant CD8+ cytotoxic T lymphocytes is discussed.

L22 ANSWER 28 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1991:918 HCAPLUS
DOCUMENT NUMBER: 114:918
TITLE: Fate of injected glucagon taken up by rat liver in vivo. Degradation of internalized ligand in the **endosomal** compartment
AUTHOR(S): Authier, Francois; Janicot, Michel; Lederer, Florence; Desbuquois, Bernard
CORPORATE SOURCE: Hop. Necker Enfants-Mal., Paris, 75015, Fr.
SOURCE: Biochemical Journal (**1990**), 272(3), 703-12
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The uptake and processing of glucagon into rat liver **endosomes** were studied in vivo by subcellular fractionation. After injection of [[125I]iodo-Tyr10]glucagon and [[125I]iodo-Tyr13]glucagon to rats, the uptake of radioactivity into the liver was maximum at 2 min (6% of the dose/g of tissue). On differential centrifugation, the radioactivity in the homogenate was recovered mainly in the nuclear (N), microsomal (P), and supernatant (S) fractions, the maximum at 5, 10, and 40 min, resp.; recovery of radioactivity in the mitochondrial-lysosomal (ML) fraction did not exceed 6% and was maximal at 20 min. On d.-gradient centrifugation, the radioactivity associated 1st (2-10 min) with plasma membranes and then (10-40 min) with Golgi-**endosomal** (GE) fractions, with 2-5-fold and 20-150-fold enrichments resp. Subfractionation of the GE fractions showed that, unlike the Golgi marker galactosyltransferase, the radioactivity was d.-shifted by diaminobenzidine cytochem. Subfractionation of the ML fraction isolated at 40 min showed that > 1/2 of the radioactivity was recovered at lower densities than the lysosomal marker acid phosphatase. Throughout the time of study, the [125I]iodoglucagon associated with the P, PM, and GE fractions remained at least 80-90% TCA-precipitable, whereas that associated with other fractions, especially the S fraction, became progressively TCA-soluble On gel filtration and HPLC, the small amount of degraded [125I]iodoglucagon associated with GE fractions consisted of monoiodotyrosine. Chloroquine treatment of [125I]iodoglucagon-injected rats caused an increase in the late recovery of radioactivity in the ML,

P, and GE fractions, but had little effect on the association of the ML radioactivity with acid-phosphatase-containing structures. Chloroquine treatment also led to a paradoxical decrease in the TCA-precipitability of the radioactivity associated with the P and GE fractions. Upon HPLC anal. of GE exts. of chloroquine-treated rats, at least 4 degradation products less **hydrophobic** than intact [125I]iodoglucagon were identified. Radio-sequence anal. of 4 of these products revealed 3 cleavages, affecting bonds Ser2-Gln3, Thr5-Phe6, and Phe6-Thr7. When GE fractions containing internalized [125I]iodoglucagon were incubated in iso-osmotic KCl at 30°, a rapid generation of TCA-soluble products was observed, with a maximum at **pH** 4. Thus, **endosomes** are a major site at which internalized glucagon is degraded, **endosomal** acidification being required for optimum degradation

L22 ANSWER 29 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:567495 HCAPLUS
DOCUMENT NUMBER: 113:167495
TITLE: Chemical properties of the anion transport inhibitory binding site of arginine-specific reagents in human red blood cell membranes
AUTHOR(S): Julien, Thomas; Betakis, Evlampios; Zaki, Laila
CORPORATE SOURCE: Max-Planck-Inst. Biophys., Frankfurt/Main, 6000/71, Germany
SOURCE: Biochimica et Biophysica Acta, Biomembranes (**1990**), 1026(1), 43-50
CODEN: BBBMBS; ISSN: 0005-2736
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A series of arginine-specific reagents with different size and polarity have been synthesized and their inhibitory potency on sulfate exchange in resealed ghosts has been investigated. The synthesized phenylglyoxal derivs. p-nitro-, p-methyl-, p-hydroxy-, p-carboxy-, p-sulfo-, and p-azido-phenylglyoxal are potent inhibitors of anion transport. The reaction between the cells and azidophenylglyoxal was performed in the dark. Exposure of the modified cells to the light was not followed by an increase in the inhibition. No crosslinking products were visible after gel electrophoresis. The rate of inactivation of sulfate flux with these reagents obeyed pseudo-first-order kinetics and increases with increasing reagents concentration and **pH.** Prolonged incubation of the cells with these reagents results in almost complete inhibition of the transport system. The pos. charged phenylglyoxal derivative 4-(trimethylaminioacetylamido)phenylglyoxal was not able to inhibit the transport system. The **hydrophobic** character and the electronic properties of these reagents do not correlate with their inhibitory potency. Their electrostatic and steric effects seem to play the major role in their action.

L22 ANSWER 30 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:545298 HCAPLUS
DOCUMENT NUMBER: 113:145298
TITLE: Mechanism of penetration and of action of local anesthetics in Escherichia coli cells
AUTHOR(S): Collura, Vincent; Letellier, Lucienne
CORPORATE SOURCE: Lab. Biomembranes, Univ. Paris Sud, Orsay, Fr.
SOURCE: Biochimica et Biophysica Acta, Biomembranes (**1990**), 1027(3), 238-44
CODEN: BBBMBS; ISSN: 0005-2736
DOCUMENT TYPE: Journal
LANGUAGE: English
AB E. coli cells were used to study the mechanism of penetration of local

anesthetics and the relationship between penetration and functional properties. The results show that both the neutral and the protonated form of dibucaine can be accumulated in the cells. Accumulation of the protonated form occurs in response to a transmembrane elec. potential (neg. inside) and results in high trapped concns. (70 mM). Accumulation can lead to an alkalinization of the internal **pH**. Low concns. of dibucaine stimulate the respiration, increase the transmembrane elec. potential and raise the accumulation of solutes. Inhibition of these functions occurs at higher concns. of the drug. Furthermore, the drug concentration required to inhibit these functions is smaller at alkaline external **pH** than at acidic external **pH**, suggesting that the inhibition is mainly due to the neutral form of the anesthetics. Other **hydrophobic** amines also stimulate and inhibit different membrane functions, their efficiency being correlated to their lipophilicity.

L22 ANSWER 31 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:51253 HCAPLUS
DOCUMENT NUMBER: 112:51253
TITLE: Growth control strength and active site of yeast plasma membrane ATPase studied by site-directed mutagenesis
AUTHOR(S): Portillo, Francisco; Serrano, Ramon
CORPORATE SOURCE: Eur. Mol. Biol. Lab., Heidelberg, Fed. Rep. Ger.
SOURCE: European Journal of Biochemistry (**1989**), 186(3), 501-7
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Several amino acids which are conserved in cation-pumping ATPases with phosphorylated intermediate have been mutagenized in the yeast plasma membrane H+-ATPase. The mutant genes have been selectively expressed in a yeast strain where the wild-type ATPase is only expressed in galactose medium. A series of mutants with decreasing levels of activity demonstrates that the ATPase is rate-limiting for growth and that decreased ATPase activity correlates with decreased intracellular **pH**. Enzymic and transport studies of mutant ATPases indicate that (a) lysine (Lys) 474 is the target for the inhibitor fluorescein 5'-isothiocyanate and this residue can be replaced by either arginine or histidine with partial retention of activity; (b) the sensitivity to inhibition by vanadate is affected by the mutations Thr231 → Gly, Cys376 → Leu, Lys379 → Gln, and Asp634 → Asn; (c) the mutation Ser234 → Ala causes uncoupling between ATP hydrolysis and proton transport and reduces the ATP content of the cells; (d) the mutation Asp730 → Asn, which affects a polar residue conserved in **hydrophobic** stretches of H+-ATPases, abolishes ATPase activity and proton transport but not the formation of a phosphorylated intermediate.

L22 ANSWER 32 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1989:626951 HCAPLUS
DOCUMENT NUMBER: 111:226951
TITLE: A point mutation of proline 308 in diphtheria toxin B chain inhibits membrane translocation of toxin conjugates
AUTHOR(S): Johnson, Virginia Gray; Youle, Richard J.
CORPORATE SOURCE: Biochem. Sect., Natl. Inst. Neurol. Dis. Stroke, Bethesda, MD, 20892, USA
SOURCE: Journal of Biological Chemistry (**1989**), 264(30), 17739-44
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal
LANGUAGE: English
AB A mutant form of diphtheria toxin (DT), CRM 102, that has a point mutation at position 308 (Pro → Ser) within one of these **hydrophobic** domains was identified. CRM 102 conjugated to a monoclonal antibody against the T cell receptor, the transferrin receptor, or transferrin itself is approx. 10-fold less toxic than native DT or a control DT mutant, CRM 103, linked to the same binding moieties. Direct measurement of membrane translocation activity by exposure of cells to low extracellular **pH** demonstrates that CRM 102 conjugates express only 10% of the translocation activity of the control toxin conjugates. However, when CRM 102 or 102 conjugates bind and kill cells via the DT receptor, no reduction in membrane translocation activity is observed The defect in CRM 102 is not evident in the presence of 20 mM NH4Cl. The defect in translocation also has no effect on the ratio of the lag time before protein synthesis inhibition begins to the rate of protein synthesis inhibition. Thus, the proline-serine substitution at position 308 disrupts the membrane translocation process and distinguishes between two routes of DT entry: DT receptor-mediated entry and entry mediated by alternate receptors.

L22 ANSWER 33 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1989:549303 HCAPLUS
DOCUMENT NUMBER: 111:149303
TITLE: Characterization and functional reconstitution of a soluble form of the **hydrophobic** membrane protein lac permease from Escherichia coli
AUTHOR(S): Roepe, Paul D.; Kaback, H. Ronald
CORPORATE SOURCE: Roche Inst. Mol. Biol., Roche Res. Cent., Nutley, NJ, 07110-1199, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (**1989**), 86(16), 6087-91
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lac permease, a polytopic membrane protein from E. coli, has been purified in soluble form by overexpressing the lacY gene by means of the T7 RNA polymerase system. Soluble permease is dissociated from membranes with urea or other chaotropes and appears after the membrane is saturated with newly synthesized permease. Remarkably, this form of the permease appears to remain soluble in phosphate buffer at neutral **pH** after removal of urea, although it aggregates in a time- and concentration-dependent manner. Importantly, soluble permease behaves as a monomer during size-exclusion chromatog. with or without urea, contains <3 mol of organic phosphate per mol of protein, and is largely helical. Soluble permease binds p-nitrophenol α-D-galactopyranoside ≈40% as well as permease in the native environment of the membrane and can be reconstituted into phospholipid vesicles that catalyze lactose counterflow or active transport in response to a membrane potential (interior neg.). The results suggest that lac permease can assume a nondenatured conformation in aqueous solution

L22 ANSWER 34 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1989:548528 HCAPLUS
DOCUMENT NUMBER: 111:148528
TITLE: Staphylococcal α-toxin: a study of membrane penetration and pore formation
AUTHOR(S): Harshman, Sidney; Boquet, Patrice; Duflot, Edith; Alouf, Joseph E.; Montecucco, Cesare; Papini, Emanuele

CORPORATE SOURCE: Sch. Med., Vanderbilt Univ., Nashville, TN, 37232, USA
SOURCE: Journal of Biological Chemistry (1989), 264(25), 14978-84
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using Controlled-Pore Glass bead-purified Staphylococcus aureus α-toxin, four events are measured as a function of **pH**: (a) release of potassium from prelabeled aolectin vesicles, (b) conversion of the toxin to a globally **hydrophobic** mol., (c) binding of detergent by the toxin, and (d) labeling of the toxin with photoactivable, radiolabeled, **hydrophobic** probes. Two of these events, potassium release and conversion to a net **hydrophobic** state, are paired in that, for the event to occur, each requires a **pH** of 4.6 or less. In contrast, photolabeling with the membrane probes PC I and PC II (where PC represents phosphatidylcholine) is easily detectable at **pH** values as high as 5.0 and 6.0. These results demonstrate that, as the **pH** is lowered, two distinct changes in the phys. properties of α-toxin occur. The first, which occurs under mild acidic conditions, converts the toxin from a water-soluble mol. into an amphipathic mol. The second, requiring relatively more acidic conditions, converts the amphipathic toxin mol. into a globally **hydrophobic** mol. Correlated with these phys. changes in the α-toxin mol. is the acquisition of two new biol. properties. The conversion of α-toxin into an amphipathic conformation correlates with the acquisition of the biol. property of the reversible penetration into the bilayer of the asolectin liposome membrane, as evidenced by labeling with the photoactivable probes. At lower **pH**, the conversion of the toxin into a globally **hydrophobic** mol. correlates with the biol. property of causing damage to the **cell membrane**, as measured by the release of internal potassium ions, presumably by the formation of transmembrane hexamer pores. Thus, penetration of the lipid bilayer by the toxin monomer is not sufficient to cause measurable membrane injury. For the release of potassium ions from asolectin vesicles, staphylococcal α-toxin demonstrably requires two separable steps: (a) penetration of the monomer into the lipid bilayer and (b) formation of an ion-permeable perturbation of the lipid membrane.

L22 ANSWER 35 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1989:71983 HCAPLUS
DOCUMENT NUMBER: 110:71983
TITLE: Azidobenzamido-008, a new photosensitive substrate for the 'multispecific bile acid transporter' of hepatocytes: evidence for a common transport system for bile acids and cyclosomatostatins in basolateral membranes
AUTHOR(S): Ziegler, Kornelia; Frimmer, Max; Kessler, H.; Haupt, A.
CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Justus-Liebig Univ., Giessen, D-6300, Fed. Rep. Ger.
SOURCE: Biochimica et Biophysica Acta, Biomembranes (**1988**), 945(2), 263-72
CODEN: BBBMBS; ISSN: 0005-2736
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cyclo(-Phe(p-NH[1-14C]Ac)-Thr-Lys-(CO(p-N3)C6H4)-Trp-Phe-D-Pro), named azidobenzamido-008, was synthesized in order to identify binding sites for cyclo(Phe-Thr-Lys-Trp-Phe-D-Pro), named 008, (a cyclosomatostatin with retro sequences) in liver cell plasma membranes. In the dark the above photolabel was taken up into isolated hepatocytes, inhibiting the

Na-dependent uptake of cholate and taurocholate in a competitive manner (Ki for cholate uptake inhibition = 1 μM; Ki for taurocholate uptake inhibition = 5 μM). When activated by flashed light the inhibition became irreversible (IC50 for cholate uptake inhibition = 2 μM; IC50 for taurocholate uptake inhibition = 9 μM) and the activated cyclopeptide bound chiefly to hepatocellular membrane proteins of 67, 54, 50, and 37 kDa. Excess of the initial 008, or of cholate or phalloidin, partially protected the above membrane components against labeling with [14C]-labeled azidobenzamido-008. In contrast AS 30 D ascites hepatoma cells, known to be deficient in bile acid and cyclosomatostatin transport, could not be specifically labeled by azidobenzamido-008. The membrane proteins preferentially labeled in hepatocytes (50 and 54 kDa) are integral glycoproteins. The 67 kDa protein is a **hydrophilic** nonglycosylated membrane component. Independent of labeling with [14C]-labeled azidobenzamido-008 or with [14C]-labeled azidobenzamido-taurocholate, the main radioactive peaks in the **pH** region of 7, 5.5, and 5.25 were identical after solubilization with Nonidet P-40 and subsequent isoelec. focusing. Proteins of 67, 54, 50, and 37 kDa could be enriched by use of 008-containing gels in affinity electrophoresis. Binding sites for 008 were not destroyed by SDS or Nonidet P-40 treatment of plasma membranes.

L22 ANSWER 36 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:416688 HCAPLUS
DOCUMENT NUMBER: 109:16688
TITLE: Membrane lipid alteration: effect on cellular uptake of mitoxantrone
AUTHOR(S): Burns, C. Patrick; Haugstad, Bradley N.; Mossman, Craig J.; North, James A.; Ingraham, Leah M.
CORPORATE SOURCE: Coll. Med., Univ. Iowa, Iowa City, IA, 52242, USA
SOURCE: Lipids (**1988**), 23(5), 393-7
CODEN: LPDSAP; ISSN: 0024-4201
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of membrane structural alteration on the cellular association of the anticancer drug mitoxantrone, whose uptake is not carrier-mediated, was studied. Membrane fatty acids of L1210 cells were modified by incubating the cells with the highly unsatd. docosahexaenoic acid (22:6), which results in isolated plasma membranes with 37% of the fatty acids as 22:6, or with the monounsatd. oleic acid (18:1), which results in 58% of the fatty acids as 18:1. The rate of uptake by 22:6-enriched cells during the 1st min was 62% greater than that by those enriched with 18:1. The higher rate was recorded at 0.5-16 μM, **pH** 6.6-7.6 and temps. 10-40°. The difference in cell-associated drug apparently was not due simply to a change in mitoxantrone solubility, as measured by partitioning of the drug in lipophilic-**hydrophilic** systems containing lipids from the fatty-acid altered cells. Apparently, the type of fatty acids contained in L1210 cell membranes can affect the cell association of mitoxantrone. This effect could be on transmembrane flux or be due to differences in binding of the drug to intracellular structures.

L22 ANSWER 37 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1986:621313 HCAPLUS
DOCUMENT NUMBER: 105:221313
TITLE: Cell damage by viruses, toxins and complement: common features of pore-formation and its inhibition by calcium
AUTHOR(S): Pasternak, C. A.; Alder, G. M.; Bashford, C. L.; Buckley, C. D.; Micklem, K. J.; Patel, K.
CORPORATE SOURCE: Dep. Biochem., St. George's Hosp. Med. Sch., London,

SW17 0RE, UK
SOURCE: Biochemical Society Symposia (1985), Volume Date 1984, 50(Mol. Basis Mov. Membr.), 247-64
CODEN: BSSYAT; ISSN: 0067-8694
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Hemolytic paramyxoviruses interact with cells in the following way: a potentially leaky viral envelope fuses with the plasma membrane, creating a **hydrophilic** pore of .apprx.1 nm in diameter; this allows ions and low-mol.-weight compds., but not proteins, to leak into and out of cells. Other viruses act similarly if the **pH** is reduced to 5. Leakage (measured by collapse of membrane potential, by movement of monovalent cations and by loss of phosphorylated intermediates from cells) is prevented by extracellular Ca2+. Ca2+ does not affect binding or fusion of virus to cells. It inhibits leakage as well as preventing it, and it aids in the recovery (i.e., the restoration of nonleakiness) of cells. Certain anti-Ca2+ drugs have an opposite effect. Expts. with the bee venom protein melittin, with the α-toxin of Staphylcoccus aureus, and with activated complement, show that the lesions produced by these agents, too, are sensitive to extracellular Ca2+ and to anti-Ca2+ drugs. The mechanisms of these effects are discussed.

L22 ANSWER 38 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1986:162715 HCAPLUS
DOCUMENT NUMBER: 104:162715
TITLE: Mutants of the membrane-binding region of Semliki Forest virus E2 protein. I. Cell surface transport and fusogenic activity
AUTHOR(S): Cutler, Daniel F.; Garoff, Henrik
CORPORATE SOURCE: Eur. Mol. Biol. Lab., Heidelberg, 6900, Fed. Rep. Ger.
SOURCE: Journal of Cell Biology (1986), 102(3), 889-901
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Three mutations of the membrane-binding region of the Semliki Forest virus (SFV) p62 polypeptide (the precursor for virion E3 and E2) were made by oligonucleotide-directed mutagenesis of a cDNA clone encoding the SFV structural proteins. One of the mutations (A2) substitutes a glutamic acid for an alanine in the middle of the **hydrophobic** stretch which spans the bilayer. A1 and A3 alter the 2 basic charged amino acids in the cytoplasmic domain next to the **hydrophobic** region. The wild-type charge cluster of Arg-Ser-Lys(+2) was changed to Gly-Ser-Met(0;A3) or to Gly-Ser-Glu(-1;A1). The mutant p62 proteins were analyzed both in the presence and the absence of E1, the other half of the heterodimer spike complex of SFV. The mutant proteins expressed in COS-7 cells are glycosylated and are of the expected sizes. When co-expressed with E1, all 3 mutants are cleaved to yield the E2 protein and transported to the surface of COS-7 cells. When expressed in the absence of E1, the mutant p62 proteins remain uncleaved but still reach the cell surface. Once at the cell surface, all 3 mutants, when co-expressed with E1, can promote low **pH**-triggered cell-cell fusion. Thus, the 3 mutant p62/E2 proteins are still membrane associated in a functionally unaltered way.

L22 ANSWER 39 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1983:120012 HCAPLUS
DOCUMENT NUMBER: 98:120012
TITLE: Vaginal absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) in rats.

III: Effect of estrous cycle on vaginal absorption of **hydrophilic** model compounds
AUTHOR(S): Okada, Hiroaki; Yashiki, Takatsuka; Mima, Hiroyuki
CORPORATE SOURCE: Cent. Res. Div., Takeda Chem. Ind., Ltd., Yodogawa, 532, Japan
SOURCE: Journal of Pharmaceutical Sciences (**1983**), 72(2), 173-6
CODEN: JPMSAE; ISSN: 0022-3549
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of estrous cycle stages on vaginal absorption in rats was determined by the use of insulin [9004-10-8], phenolsulfophthalein [143-74-8], and salicylic acid [69-72-7] as **hydrophilic** model compds. Absorption of these compds. was marked affected by the stage, possibly due to the change of transport rate through the pore-like pathways. The absorption of phenolsulfonphthalein during proestrus and estrus is roughly 0.1 of that during metestrus and diestrus. An increase of the nonionized form of salicylic acid, produced by a lowered **pH**, resulted in an enhancement of absorption during proestrus and diestrus; higher contribution of the transport through the **cell membrane** possibly reduced an effect of the estrous cycle. However, consecutive daily administration of leuprolide [53714-56-0] halted the cycle at diestrus and reduced the cycle effect on the vaginal absorption of phenolsulfonphthalein; when the treatment was started at any of the 4 stages of the cycle, vaginal absorption was enhanced .apprx.20%, with less variance than that observed in normal diestrous rats.

L22 ANSWER 40 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1981:561845 HCAPLUS
DOCUMENT NUMBER: 95:161845
TITLE: Time dependence of the effect of p-chloromercuribenzoate on erythrocyte water permeability: a pulsed nuclear magnetic resonance study
AUTHOR(S): Ashley, David L.; Goldstein, J. H.
CORPORATE SOURCE: Dep. Chem., Emory Univ., Atlanta, GA, 30322, USA
SOURCE: Journal of Membrane Biology (**1981**), 61(3), 199-207
CODEN: JMBBBO; ISSN: 0022-2631
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Pulsed NMR spectroscopy is employed to determination the time dependence of the change in erythrocyte H2O permeability following exposure to p-chloromercuribenzoate (PCMB) [138-85-2] or p-chloromercuribenzene sulfonic acid (PCMBS) [554-77-8]. PCMB reacted with at least 2 sulfhydryl groups which affect water permeability. This was shown by the double exponential character of the change in erythrocyte diffusional permeability with time after PCMB addition However, only one inhibition rate process could be distinguished following PCMBS exposure, suggesting that 1 site bound by PCMB is not accessible to PCMBS. This site is postulated to be located in a **hydrophobic** region of the membrane, whereas the site reached by both drugs is located in the normal anion permeation channel. The effect of **pH** on the degree of inhibition due to each component and the inhibition rates is explained in terms of its effect on solubility of the reagents in the membrane and variation of the dissociated-to-undissocd. ratio of PCMB.

L22 ANSWER 41 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1979:401468 HCAPLUS
DOCUMENT NUMBER: 91:1468

TITLE: Characterization of glutamate transport system in **hydrophobic** protein (H protein) of Bacillus subtilis
AUTHOR(S): Kusaka, Iwao; Kanai, Keiko
CORPORATE SOURCE: Inst. Appl. Microbiol., Univ. Tokyo, Tokyo, Japan
SOURCE: Biochimica et Biophysica Acta, Biomembranes (**1979**), 552(3), 492-8
CODEN: BBBMBS; ISSN: 0005-2736
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Hydrophobic** protein (H protein) was isolated from membrane fractions of B. subtilis and constituted into artificial membrane vesicles with lipid of B. subtilis. Glutamate was accumulated by the vesicle when a Na+ gradient across the membrane was imposed. The maximum effect of Na+ on transport was observed at .apprx.40 mM Na+, whereas the apparent Km for Na+ was .apprx.8 mM. The Km for glutamate in the presence of 50 mM Na+ was .apprx.8 μM. Increasing the concentration of Na+ resulted in a decrease in the Km for glutamate; the Vmax was not affected. The transport was sensitive to monensin (Na+ ionophore). Glutamate was also accumulated when the **pH** gradient (interior alkaline) across the membrane was imposed or a membrane potential was induced with a K+ diffusion potential. The **pH** gradient-driven glutamate transport was sensitive to carbonylcyanide m-chlorophenylhydrazone and the apparent Km for glutamate was .apprx.25 μM. Thus, there are 2 glutamate transport systems present in H protein; one is Na+-dependent and the other is H+-dependent.

L22 ANSWER 42 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1979:163642 HCAPLUS
DOCUMENT NUMBER: 90:163642
TITLE: Proteolytic studies of chain cleavage and proton pump activity of bacteriorhodopsin in purple membranes
AUTHOR(S): Tsuji, Kinko; Rosenheck, Kurt
CORPORATE SOURCE: Dep. Membrane Res., Weizmann Inst. Sci., Rehovot, Israel
SOURCE: Bioelectrochemistry and Bioenergetics (**1978**), 5(4), 723-40
CODEN: BEBEBP; ISSN: 0302-4598
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cleavage of bacteriorhodopsin in purple membranes of Halobacterium halobium by proteolytic digestion led to fragments, the mol. wts. of which depended on the enzyme used. On Na dodecyl sulfate-polyacrylamide gels, trypsin gave bands corresponding to 23,000 and 22,000 daltons. Pronase-derived peptides had mol. wts. of 22,000 and 17,400 daltons, and papain-derived peptides had mol. wts. of 21,000 and 16,600 daltons, when enzyme treatment was carried out in the dark. When purple membranes were illuminated during Pronase digestion, an addnl. small fragment with a mol. weight of .apprx.800 and consisting of primarily of **hydrophobic** amino acids was split off. The kinetics of the light-induced **pH** changes in suspensions of reconstituted proteoliposomes incorporating enzymically modified bacteriorhodopsin was fitted by two 1st-order processes, 1 being .apprx.20-fold faster than the other. After trypsin treatment the kinetics were not significantly changed. Pronase treatment drastically reduced the light-induced **pH** changes, acting mainly on the amplitude of the slower processes. The apparent rate consts. of both processes were markedly increased. Similar, but less drastic effects occurred in papain-treated membranes. The increases in the rate consts. of the slow phase can be accounted for by assuming that the proton leak through the proteoliposome membrane is increased by the proteolytic cleavage of the bacteriohodopsin chain.

L22 ANSWER 43 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1975:120251 HCAPLUS
DOCUMENT NUMBER: 82:120251
TITLE: Studies on the mechanism and reversal of the phospholipase-A2 inactivation of D-glucose uptake by isolated human erythrocyte membranes
AUTHOR(S): Banjo, Batya; Walker, Caroline; Rohrlick, Ruth; Kahlenberg, Arthur
CORPORATE SOURCE: Lady Davis Inst. Med. Res., Jewish Gen. Hosp., Montreal, QC, Can.
SOURCE: Canadian Journal of Biochemistry (**1974**), 52(12), 1097-109
CODEN: CJBIAE; ISSN: 0008-4018
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The mechanism underlying the inactivation of the stereospecific uptake of D-glucose by isolated human-erythrocyte membranes following digestion with phospholipase A2 (Kahlenberg, A.; Banjo, B., 1972) was investigated. This inactivation was not accompanied by any significant change in the uptake of L-glucose. The decrease in D-glucose uptake following limited (25-30%) cleavage of membrane phospholipids by phospholipase A2 was characterized by a 2-fold increase in the apparent dissociation constant of the D-glucose-membrane complex and a 34% decrease in the membrane's maximum capacity for D-glucose uptake. These effects of phospholipase A2 were completely reversed upon removal of the membrane-bound phospholipid by-products (fatty acids and lysophospholipids) by washing the membranes wih defatted bovine serum albumin. Oleic acid and various lysophosphatides added to albumin-washed, phospholipase A2-treated membranes in amts. formed by the enzyme treatment produced negligible inhibition of D-glucose uptake. With more extensive phosholipase A2 digestion of membrane phospholipids, defatted bovine serum albumin did not restore D-glucose uptake despite the removal of the phospholipid by-products formed. In addition to the inactivation of D-glucose uptake, limited enzyme treatment transformed the appearance of the membranes collected by centrifugation from opaque white to transparent and gelatinous. Both of these effects of phospholipase A2 were completely reversed upon incubation of the membranes at **pH** 5.5 for 2 hr at 37° without loss of any of the membrane lysophosphatides and fatty acids formed by the enzyme treatment. Apparently, this **pH**- and temperature-dependent restoration of D-glucose uptake is due to a conformational change resulting in the relocation of the membrane D-glucose-binding sites into a functional environment. Thus, the inactivation of D-glucose uptake by phospholipase A2, which was not accompanied by any change of L-glucose uptake, occurs by 2 different mechanisms. With limited hydrolysis of membrane phosholipids, one or both of the resulting phospholipid by-products reversibly inhibit the uptake of D-glucose by decreasing the affinity of the membrane for D-glucose and by masking a portion of the total available D-glucose-binding sites. However, upon extensive cleavage of phospholipids in the **hydrophobic** region of the membrane, there is an apparently irreversible disorganization of the membrane D-glucose-binding component. This might be due to destruction of vital phospholipids and(or) a disturbance of the interactions between the lipid and protein components of the membrane.

L22 ANSWER 44 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1975:81212 HCAPLUS
DOCUMENT NUMBER: 82:81212
TITLE: Effect of phloretin on red cell nonelectrolyte

permeability
AUTHOR(S): Owen, Jeffrey D.; Steggall, Mary; Eyring, Edward M.
CORPORATE SOURCE: Dep. Physiol., Univ. Utah, Salt Lake City, UT, USA
SOURCE: Journal of Membrane Biology (**1974**), 19(1-2), 79-92
CODEN: JMBBBO; ISSN: 0022-2631
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effect of phloretin [60-82-2] on permeability of small nonelectrolytes into human red cells was bimodal for **hydrophilic** mols. and nonbimodal for lipophilic mols. At low phloretin concns. (<0.1 mM) **hydrophilic** as well as lipophilic nonelectrolyte permeation was increased. At high phloretin concns (>0.1 mM) the permeability of **hydrophilic** mols. was decreased, whereas lipophilic mol. permeability continued to be increased. The mechanism for phloretin acting on red cell nonelectrolyte pathways is apparently different for **hydrophilic** than for lipophilic permeant mols. The keto or un-ionized form of phloretin (present at low **pH**), which is known to have a greater affinity for the membrane, had a larger effect on **hydrophilic** nonelectrolyte permeability than did the ionized form of phloretin.

L22 ANSWER 45 OF 45 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1974:46194 HCAPLUS
DOCUMENT NUMBER: 80:46194
TITLE: Effect of physicochemical parameters on the red **cell membrane** permeability constant for anions
AUTHOR(S): Gomulkiewicz, Jan
CORPORATE SOURCE: Akad. Roln., Wroclaw, Pol.
SOURCE: Zeszyty Naukowe Akademii Rolniczej we Wroclawiu, Weterynaria (**1973**) 7-47
CODEN: ZNRWA9; ISSN: 0137-1975
DOCUMENT TYPE: Journal
LANGUAGE: Polish
AB The effect of ionic strength, **pH**, types of ions involved, and temperature on the anion permeability of erythrocyte membranes was studied with the aid of PO43- and SO42- anions labeled with 32P and 35S, resp. A decrease of ionic strength and **pH** of the medium was accompanied by an increase in the permeability constant of anion penetration through erythrocyte **cell membrane.** The decrease in ionic strength resulted also in a decrease of the activation energy of the anion permeation. The decrease of elec. charge of the membranes in the presence of neuraminidase, methylene blue, or Mg2+ changed the permeability constant considerably. The energy barrier for anion permeation is apparently related to the **hydrophobic** layer of the membrane. Any changes in the permeability are caused by structural changes of this layer due to changes in phys.-chemical properties of the medium.

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=> d que stat l27
L8         26508 SEA FILE=HCAPLUS ABB=ON  BIOLOGICAL TRANSPORT+ALL AND CELL
                 MEMBRANE+ALL
L10          736 SEA FILE=HCAPLUS ABB=ON  L8 AND (?HYDROPHOB? OR ?HYDROPHIL?)
L11           68 SEA FILE=HCAPLUS ABB=ON  L10 AND PH
L12            2 SEA FILE=HCAPLUS ABB=ON  L10 AND PH(3A)?SENSITIV?
L15            1 SEA FILE=HCAPLUS ABB=ON  L10 AND ?VINYL?
L16            5 SEA FILE=HCAPLUS ABB=ON  L11 AND (?ENDOSOM? OR ?ENDOCYT?)
L18            4 SEA FILE=HCAPLUS ABB=ON  L10 AND (?POLYALK?(W)?OXID? OR
                 ?POLYOXYALKYLENE?)
L19           72 SEA FILE=HCAPLUS ABB=ON  L11 OR L12 OR L15 OR L16 OR L18
L24          176 SEA L19
L27            9 SEA L24 AND (?THERAP? OR ?DIAG?)

=> d ibib abs l27 1-9

L27  ANSWER 1 OF 9     MEDLINE on STN
ACCESSION NUMBER:   2005346124     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 15997889
TITLE:              Cell-penetrating peptides: [corrected] from inception to
                    application.
AUTHOR:             Magzoub Mazin; Graslund Astrid
CORPORATE SOURCE:   Department of Biochemistry and Biophysics, The Arrhenius
                    Laboratories, Stockholm University, S- 106 91 Stockholm,
                    Sweden.
SOURCE:             Quarterly reviews of biophysics, (2004 May) Vol. 37, No. 2,
                    pp. 147-95.  Ref: 384
                    Journal code: 0144032. ISSN: 0033-5835.
PUB. COUNTRY:       England: United Kingdom
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
                    General Review; (REVIEW)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200508
ENTRY DATE:         Entered STN: 7 Jul 2005
                    Last Updated on STN: 31 Aug 2005
                    Entered Medline: 30 Aug 2005
AB   Despite continuing advances in the development of macromolecules,
     including peptides, proteins, and oligonucleotides, for
     therapeutic purposes, the successful application of these
     hydrophilic molecules has so far been hampered by their inability
     to efficiently traverse the cellular plasma membrane.  The discovery of a
     class of peptides (cell-penetrating peptides, CPPs) with the ability to
     mediate the non-invasive and efficient import of a whole host of cargoes,
     both in vitro and in vivo, has provided a new means by which the problem
     associated with cellular delivery can be circumvented.  A complete
     understanding of the translocation mechanism(s) of CPPs has so far proven
     elusive.  Initial studies indicated an ATP-independent, non-
     endocytotic mechanism, dependent on direct peptide-membrane
     interactions, making it an enticing challenge from a biophysical point of
     view.  However, recent evidence cast doubt on many of the earlier results,
     and led to a re-evaluation of the translocation mechanism of CPPs.  In
     this review a brief history of the field will be given, followed by an
     introduction to some of the better known and more widely used CPPs,
     including some of their current applications, and finally a discussion of
     the translocation mechanism(s) and the controversies surrounding it.

L27  ANSWER 2 OF 9     MEDLINE on STN
ACCESSION NUMBER:   2003160519     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 12676459
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TITLE: The role of multidrug transporters in drug availability, metabolism and toxicity.
AUTHOR: Bodo Adrienn; Bakos Eva; Szeri Flora; Varadi Andras; Sarkadi Balazs
CORPORATE SOURCE: National Medical Center, Institute of Haematology and Immunology, Membrane Research Group of the Hungarian Academy of Sciences, Budapest, Hungary.
SOURCE: Toxicology letters, (2003 Apr 11) Vol. 140-141, pp. 133-43. Ref: 36
Journal code: 7709027. ISSN: 0378-4274.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 6 Apr 2003
Last Updated on STN: 8 May 2003
Entered Medline: 7 May 2003

AB Multidrug resistance is frequently observed when treating cancer patients with **chemotherapeutic** agents. A variety of ATP binding cassette (ABC) transporters, localized in the **cell membrane**, cause this phenomenon by extruding a variety of **chemotherapeutic** agents from the tumor cells. However, the major physiological role of the multidrug transporters is the protection of our cells and tissues against xenobiotics, and these transporters play a key role in drug availability, metabolism and toxicity. Three major groups of ABC transporters are involved in multidrug resistance: the classical P-glycoprotein MDR1, the multidrug resistance associated proteins (MRP1, MRP2, and probably MRP3, MRP4 and MRP5), and the ABCG2 protein, an ABC half-transporter. All these proteins were shown to catalyze an ATP-dependent active transport of chemically unrelated compounds. MDR1 (P-glycoprotein) and ABCG2 preferentially extrude large **hydrophobic**, positively charged molecules, while the members of the MRP family can extrude both **hydrophobic** uncharged molecules and water-soluble anionic compounds. By examining the interactions of the multidrug transporters with pharmacological and toxic agents, a prediction for the cellular and tissue distribution of these compounds can be achieved. Oral bioavailability, entering the blood-brain and blood-CSF barrier, reaching the fetus through the placenta, liver and kidney secretion, cellular entry for affecting intracellular targets, are all questions, which can be addressed by basic in vitro studies on the multidrug resistance proteins. Investigation of the substrate interactions and modulation of multidrug transporters may pave the way for predictive toxicology and pharmacogenomics. Here we show that by using in vitro assay systems it is possible to measure the interactions of multidrug transporters with various drugs and toxic agents. We focus on the characterisation of the MRP1 and MRP3 proteins, their relevance in chemoresistance of cancer and in drug metabolism and toxicity.

L27 ANSWER 3 OF 9 MEDLINE on STN
ACCESSION NUMBER: 2002698660 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12244102
TITLE: A novel electron paramagnetic resonance approach to determine the mechanism of drug transport by P-glycoprotein.
AUTHOR: Omote Hiroshi; Al-Shawi Marwan K
CORPORATE SOURCE: Department of Molecular Physiology and Biological Physics, University of Virginia Health System, Charlottesville, Virginia 22908-0736, USA.

CONTRACT NUMBER: GM52502 (NIGMS)
SOURCE: The Journal of biological chemistry, (2002 Nov 22) Vol. 277, No. 47, pp. 45688-94. Electronic Publication: 2002-09-19.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 17 Dec 2002
Last Updated on STN: 8 Jan 2003
Entered Medline: 7 Jan 2003

AB ATP-driven pumping of a variety of drugs out of cells by the human P-glycoprotein poses a serious problem to medical **therapy**. High level heterologous expression of human P-glycoprotein, in the yeast Saccharomyces cerevisiae, has facilitated biophysical studies in purified proteoliposome preparations. Membrane permeability of transported drugs and consequent lack of an experimentally defined drug position have made resolution of the transport mechanism difficult by classical techniques. To overcome these obstacles we devised a novel EPR spin-labeled verapamil for use as a transport substrate. Spin-labeled verapamil was an excellent transport substrate with apparent turnover number, K(m) and K(i) values of 5.8 s(-1), 4 microm, and 210 microm, respectively, at **pH** 7.4 and 37 degrees C. The apparent affinities were approximately 10-fold higher than for unlabeled verapamil. Spin-labeled verapamil stimulated ATPase activity approximately 5-fold, was relatively **hydrophilic**, and had a very low flip-flop rate, making it an ideal transport substrate. The K(m) for MgATP activation of transport was 0.8 mm. By measuring the mobility of spin-labeled verapamil during transport experiments, we were able to resolve the location of the drug in proteoliposome suspensions. Steady state gradients of spin-labeled verapamil within the range of K(i)/K(m) ratios were observed.

L27 ANSWER 4 OF 9 MEDLINE on STN
ACCESSION NUMBER: 1998228609 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9567214
TITLE: P glycoprotein: a new mechanism to control drug-induced nephrotoxicity.
AUTHOR: del Moral R G; Olmo A; Aguilar M; O'Valle F
CORPORATE SOURCE: Department of Pathology, University Hospital and School of Medicine, University of Granada, Spain.
SOURCE: Experimental nephrology, (1998 Mar-Apr) Vol. 6, No. 2, pp. 89-97. Ref: 59
Journal code: 9302239. ISSN: 1018-7782.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 18 Jun 1998
Last Updated on STN: 18 Jun 1998
Entered Medline: 8 Jun 1998

AB The role of P glycoprotein (P-gp) in kidney is now being explored, and under physiological conditions, this protein is thought to be an excretory pump of cationic xenobiotics and metabolites. Functionally, two different types of P-gp have been described, but only the class I has been related to drug transport, and its overexpression confers the multidrug resistance phenotype in tumoral cells. It has been proposed that P-gp is involved in

the energy-dependent transport of substrates through the **cell membrane** (toxic metabolites, toxins, nutrients, ions, peptides, etc.)--like a '**hydrophobic** molecule vacuum cleaner'. Several physiological functions have been attributed to P-gp: defense against xenobiotic aggression and transmembrane transport of prenylcysteine methyl esters, removing these cytotoxic metabolites from cells. A variety of substrates ranging from **chemotherapeutics** to steroid hormones, antibiotics, and calcium channel blockers can be transported by P-gp, suggesting the possible involvement of this protein in other unknown functions. Results from our group and others have suggested that overexpression of P-gp in renal tubular and mesangial cells prevents pharmacological nephrotoxicity by cyclosporin A (CsA). On the other hand CsA, a substrate of the pump, could act as a blocker in tubular cells by competitive inhibition. One relevant aspect in kidney is the possible relationship between P-gp and protein kinase C. Several reports suggest that protein kinase C may play a role in inducing the P-gp overexpression in cells under xenobiotic pressure, through activation of the ras oncoprotein family. This could be mediated directly by angiotensin II as a ras activator. This way, the detoxicant function of P-gp against products of the ras catabolism could mediate their accumulation when the 'vacuum cleaner' function is blocked by CsA or tacrolimus, contributing to the initial development of fibroblastic activation that leads to interstitial fibrosis associated with nephrotoxicity by these immunosuppressor drugs. In conclusion, P-gp expression could be an important component of a complex detoxifying system in kidney against xenobiotics or regulating the traffic of endogenous metabolites responsible for the susceptibility of subjects to the development of nephrotoxicity against different drugs.

L27 ANSWER 5 OF 9 MEDLINE on STN
ACCESSION NUMBER: 97366550 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9223380
TITLE: Permeability and metabolic properties of a trophoblast cell line (HRP-1) derived from normal rat placenta.
AUTHOR: Shi F; Soares M J; Avery M; Liu F; Zhang X; Audus K L
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence 66047, USA.
CONTRACT NUMBER: N01DA-4-7405 (NIDA)
SOURCE: Experimental cell research, (1997 Jul 10) Vol. 234, No. 1, pp. 147-55.
Journal code: 0373226. ISSN: 0014-4827.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 13 Aug 1997
Last Updated on STN: 6 Feb 1998
Entered Medline: 1 Aug 1997

AB The HRP-1 cell line is derived from normal rat placenta and appears morphologically similar to and retains characteristic expression of cellular markers of labyrinthine trophoblast cells. In this study, monolayers of HRP-1 cells grown on permeable supports were evaluated as a potential in vitro system to study trophoblast transport and metabolism. The cell line was shown to express and retain functional activity of the predominant placental cytochrome P450 isozyme, CYP1A1. Additionally, the HRP-1 cells retain functional activity of angiotensin I converting enzyme and carboxypeptidase N-like enzyme, peptidases characteristic of the trophoblast. The permeation of several **hydrophilic**, inert markers across the HRP-1 monolayers was observed to be dependent on

effective molecular size and to be passive in nature. Functional asymmetry of the HRP-1 cells was illustrated by the predominant permeation of linoleic acid in the apical-to-basolateral direction across the monolayers. Transferrin passage across HRP-1 monolayers was concentration-dependent, was bidirectional, and could be inhibited by unlabeled transferrin, features typical of the trophoblast transport system for transferrin. Collectively, these properties suggest that the HRP-1 cell line may provide a useful tool for evaluating some of the permeability and metabolic properties of the trophoblast.

L27 ANSWER 6 OF 9 MEDLINE on STN
ACCESSION NUMBER: 93217704 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1297801
TITLE: Membrane transport of folate compounds.
AUTHOR: Huennekens F M; Vitols K S; Pope L E; Fan J
CORPORATE SOURCE: Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, California 92037.
CONTRACT NUMBER: CA-39836 (NCI)
SOURCE: Journal of nutritional science and vitaminology, (1992) Vol. Spec No, pp. 52-7. Ref: 17
Journal code: 0402640. ISSN: 0301-4800.
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 21 May 1993
Last Updated on STN: 3 Feb 1997
Entered Medline: 4 May 1993

AB All eukaryotic cells and some prokaryotes that are unable to synthesize folic acid utilize membrane-associated transport systems for acquisition of the pre-formed vitamin or its coenzyme forms from external sources. These transport systems, in addition to providing folates essential for cell replication, are also important because of their role in the internalization of antifolates such as Methotrexate (MTX) that are used extensively in cancer **chemotherapy.** Information about the components and mechanism of folate transport systems has been derived, in large part, from studies with Lactobacillus casei and L1210 mouse leukemia cells, which serve as convenient models for prokaryotes and eukaryotes, respectively. L. casei contain a single folate transport system whose Kt value (i.e., concentration for half-maximum rate of uptake) for the preferred substrate folate is in the nanomolar range. The **hydrophobic** membrane-associated folate transport protein (18 kDa) has been purified to homogeneity and characterized. Expression of this transporter is repressed in cells grown on high concentrations (microM) of folate. L1210 cells contain two separate transport systems for folate compounds: (1) the low affinity system (Kt values for the preferred substrates 5-methyl- and 5-formyltetrahydrofolate and MTX in the microM range); and (2) the high affinity system (Kt for folate in the nM range). Fluorescein and biotin derivatives of MTX and folate, after conversion to N-hydroxysuccinimide esters, can be attached covalently to the transporters. These probes have been used for visualizing the transporters by fluorescence and electron microscopy and for their purification to homogeneity. (ABSTRACT TRUNCATED AT 250 WORDS)

L27 ANSWER 7 OF 9 MEDLINE on STN
ACCESSION NUMBER: 93203273 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8454643
TITLE: Mutagenesis of conserved residues in the phosphorylation

domain of the yeast plasma membrane H(+)-ATPase. Effects on structure and function.
AUTHOR: Rao R; Slayman C W
CORPORATE SOURCE: Department of Genetics, Yale University School of Medicine, New Haven, Connecticut 06510.
CONTRACT NUMBER: GM 15761 (NIGMS)
SOURCE: The Journal of biological chemistry, (1993 Mar 25) Vol. 268, No. 9, pp. 6708-13.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199304
ENTRY DATE: Entered STN: 7 May 1993
Last Updated on STN: 7 May 1993
Entered Medline: 22 Apr 1993

AB A **diagnostic** feature of P-ATPases is a phosphorylation motif (DKTGTLT), located in the **hydrophilic** center of the polypeptide chain, within which the beta-aspartyl-phosphate reaction intermediate is formed. The roles of four invariant residues (Lys379, Thr380, Thr382, and Thr384) in this region of the yeast plasma membrane H(+)-ATPase have been analyzed by site-directed mutagenesis. In addition, a set of six insertion mutants was generated containing a single glycine residue at each of the indicated sites: [sequence: see text] C S D K T GT LT to examine spatial arrangements within this highly conserved domain. In order to minimize toxic effects of the mutations on cell growth, the defective ATPases were expressed behind an inducible heat shock promoter and targeted to an intracellular pool of secretory vesicles, while wild-type ATPase was maintained in the plasma membrane where it is required for viability. Secretory vesicles containing mutant ATPase were isolated as described previously (Nakamoto, R. K., Rao, R., and Slayman, C. W. (1991) J. Biol. Chemical 266, 7940-7949) and assayed for the amount of ATPase polypeptide and for rates of ATP hydrolysis and H+ pumping. All of the insertion mutations led to biosynthetic arrest of the defective enzyme, with no ATPase appearing in the secretory vesicles. Nonconservative amino acid substitutions (Lys-->Gln, Thr-->Ala) inactivated the ATPase, whereas conservative substitutions (Lys-->Arg, Thr-->Ser) retained partial activity which has been characterized in detail. There was little or no change in the Km for ATP or the **pH** optimum in any of the mutant enzymes. Strikingly, however, all displayed an increase in resistance to vanadate, consistent with the idea that the residues in question contribute to a phosphate/vanadate binding site or that they affect the equilibrium between E1 and E2 conformations of the enzyme.

L27 ANSWER 8 OF 9 MEDLINE on STN
ACCESSION NUMBER: 89141729 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2852254
TITLE: Amiloride and its analogs as tools in the study of ion transport.
AUTHOR: Kleyman T R; Cragoe E J Jr
CORPORATE SOURCE: Department of Medicine, Columbia University, New York, New York 10032.
CONTRACT NUMBER: AM34742 (NIADDK)
SOURCE: The Journal of membrane biology, (1988 Oct) Vol. 105, No. 1, pp. 1-21. Ref: 86
Journal code: 0211301. ISSN: 0022-2631.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198903
ENTRY DATE: Entered STN: 6 Mar 1990
Last Updated on STN: 3 Feb 1997
Entered Medline: 24 Mar 1989

AB Amiloride inhibits most plasma membrane Na+ transport systems. We have reviewed the pharmacology of inhibition of these transporters by amiloride and its analogs. Thorough studies of the Na+ channel, the Na+/H+ exchanger, and the Na+/Ca2+ exchanger, clearly show that appropriate modification of the structure of amiloride will generate analogs with increased affinity and specificity for a particular transport system. Introduction of **hydrophobic** substituents on the terminal nitrogen of the guanidino moiety enhances activity against the Na+ channel; whereas addition of **hydrophobic** (or **hydrophilic**) groups on the 5-amino moiety enhances activity against the Na+/H+ exchanger. Activity against the Na+/Ca2+ exchanger and Ca2+ channel is increased with **hydrophobic** substituents at either of these sites. Appropriate modification of amiloride has produced analogs that are several hundred-fold more active than amiloride against specific transporters. The availability of radioactive and photoactive amiloride analogs, anti-amiloride antibodies, and analogs coupled to support matrices should prove useful in future studies of amiloride-sensitive transport systems. The use of amiloride and its analogs in the study of ion transport requires a knowledge of the pharmacology of inhibition of transport proteins, as well as effects on enzymes, receptors, and other cellular processes, such as DNA, RNA, and protein synthesis, and cellular metabolism. One must consider whether the effects seen on various cellular processes are direct or due to a cascade of events triggered by an effect on an ion transport system.

L27 ANSWER 9 OF 9 MEDLINE on STN
ACCESSION NUMBER: 87302537 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3304754
TITLE: How do non-steroidal anti-inflammatory drugs affect gastric mucosal defenses?.
AUTHOR: Fromm D
SOURCE: Clinical and investigative medicine. Medecine clinique et experimentale, (1987 May) Vol. 10, No. 3, pp. 251-8. Ref: 55
Journal code: 7804071. ISSN: 0147-958X.
PUB. COUNTRY: Canada
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 5 Mar 1990
Last Updated on STN: 5 Mar 1990
Entered Medline: 20 Oct 1987

AB The gastric mucosa possesses a number of mechanisms permitting resistance to damage from its own secreted acid. No single mechanism can account for gastric mucosal defense. Mucosal permeability to acid, active ion transport, blood flow, mucus secretion, epithelial restitution, and prostaglandin synthesis are among the multiple factors involved in gastric mucosal defense. Non-steroidal anti-inflammatory drugs (NSAIDs) cause gross mucosal damage by affecting these defenses. The net effect of NSAIDs is to make the mucosa more susceptible to the damaging effects of acid in the lumen. Acid plays a dual role in this process, by increasing

drug absorption (which in turn increases mucosal permeability) and by diffusing from the lumen into the mucosa. If a sufficient amount of acid entering the tissue is unbuffered, necrosis occurs. NSAIDs affect tissue bicarbonate in several ways. These drugs decrease cellular production and secretion of bicarbonate, but increase tissue entry of bicarbonate from blood. NSAIDs also have a dual effect on blood flow. Microvascular stasis occurs at sites of gross mucosal damage, but blood flow increases at visibly normal sites. Mucus is impermeable to pepsin, slows acid diffusion to some degree, traps bicarbonate to create an alkaline interface, and traps cell slough, forming another putative barrier. NSAIDs inhibit mucus secretion and modify its structure. Perhaps related to mucus is the **hydrophobic** property of the mucosa attributable to an absorbed layer of surfactant. Aspirin reduces surface **hydrophobicity**, an effect that may increase ion permeability. In addition to secreting mucus, the cells lining the luminal surface also play a key role in maintaining the permeability and active transport properties of the mucosa.(ABSTRACT TRUNCATED AT 250 WORDS)

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=> d his ful

     (FILE 'HOME' ENTERED AT 11:08:31 ON 11 AUG 2006)

     FILE 'HCAPLUS' ENTERED AT 11:09:10 ON 11 AUG 2006
               E HOFFMAN ALLAN S/AU
L1          362 SEA ABB=ON  ("HOFFMAN ALLAN S"/AU OR "HOFFMAN ALLAN SACHS"/AU
                OR "HOFFMAN ALLEN"/AU OR "HOFFMAN ALLEN H"/AU OR "HOFFMAN
                ALLEN SACHS"/AU)
               E STAYTON PATRICK S/AU
L2          177 SEA ABB=ON  ("STAYTON PAT S"/AU OR "STAYTON PATRICK"/AU OR
                "STAYTON PATRICK S"/AU OR "STAYTON PATRICK SEAN"/AU)
               E MURTHY NIREN/AU
L3           38 SEA ABB=ON  ("MURTHY NIRANJANA"/AU OR "MURTHY NIREN"/AU)
L4           16 SEA ABB=ON  L1 AND L2 AND L3
L5            8 SEA ABB=ON  L4 AND ?MEMBRANE?(W)?DISRUPT?
L6            4 SEA ABB=ON  L5 AND ?TRANSPORT?
L7              ANALYZE L5 1-8 CT :       60 TERMS
L8        26508 SEA ABB=ON  BIOLOGICAL TRANSPORT+ALL AND CELL MEMBRANE+ALL
L9           87 SEA ABB=ON  L8 AND ?HYDROPHOB? AND ?HYDROPHIL?
L10         736 SEA ABB=ON  L8 AND (?HYDROPHOB? OR ?HYDROPHIL?)
L11          68 SEA ABB=ON  L10 AND PH
L12           2 SEA ABB=ON  L10 AND PH(3A)?SENSITIV?
L13           0 SEA ABB=ON  L11 AND ?VINYL?(3A)(?COMPOUND? OR ?COMPD? OR
                ?POLYMER?)
L14           0 SEA ABB=ON  L11 AND ?VINYL?
L15           1 SEA ABB=ON  L10 AND ?VINYL?
L16           5 SEA ABB=ON  L11 AND (?ENDOSOM? OR ?ENDOCYT?)
L17           0 SEA ABB=ON  L11 AND (?POLYALK?(W)?OXID? OR ?POLYOXYALKYLENE?)
L18           4 SEA ABB=ON  L10 AND (?POLYALK?(W)?OXID? OR ?POLYOXYALKYLENE?)
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L20          45 SEA ABB=ON  L19 AND (PRD<20000107 OR PD<20000107)
L21           1 SEA ABB=ON  L20 AND (?THERAP? OR ?DIAG?)
L22          45 SEA ABB=ON  L20 OR L21   45 cits from CAPlus
L23           0 SEA ABB=ON  L22 AND PH(W)?SENSITIV?

     FILE 'MEDLINE, BIOSIS, EMBASE, JAPIO, JICST-EPLUS' ENTERED AT 11:28:26 ON
     11 AUG 2006
L24         176 SEA ABB=ON  L19
L25           0 SEA ABB=ON  L24 AND ?VINYL?
L26           0 SEA ABB=ON  L24 AND (?POLYALK?(W) ?OXID? OR ?POLYOXYALKYLENE?)
L27           9 SEA ABB=ON  L24 AND (?THERAP? OR ?DIAG?)   9 cits from above d.b.'s

     FILE 'USPATFULL' ENTERED AT 12:07:15 ON 11 AUG 2006
L28           0 SEA ABB=ON  L20 OR L21

     FILE HOME

     FILE HCAPLUS

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FILE COVERS 1907 - 11 Aug 2006 VOL 145 ISS 8
FILE LAST UPDATED: 10 Aug 2006 (20060810/ED)

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FILE MEDLINE
FILE LAST UPDATED: 10 Aug 2006 (20060810/UP). FILE COVERS 1950 TO DATE.

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The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>). See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE BIOSIS
FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 9 August 2006 (20060809/ED)

FILE EMBASE
FILE COVERS 1974 TO 11 Aug 2006 (20060811/ED)

EMBASE has been reloaded. Enter HELP RLOAD for details.

EMBASE is now updated daily. SDI frequency remains weekly (default) and biweekly.

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE JAPIO
FILE LAST UPDATED: 3 APR 2006 <20060403/UP>
FILE COVERS APRIL 1973 TO DECEMBER 22, 2005

>>> GRAPHIC IMAGES AVAILABLE <<<

>>> NEW IPC8 DATA AND FUNCTIONALITY NOT YET AVAILABLE IN THIS FILE. USE IPC7 FORMAT FOR SEARCHING THE IPC. WATCH THIS SPACE FOR FURTHER DEVELOPMENTS AND SEE OUR NEWS SECTION FOR FURTHER INFORMATION ABOUT THE IPC REFORM <<<

FILE JICST-EPLUS
FILE COVERS 1985 TO 7 AUG 2006 (20060807/ED)

THE JICST-EPLUS FILE HAS BEEN RELOADED TO REFLECT THE 1999 CONTROLLED TERM (/CT) THESAURUS RELOAD.

FILE WPIDS
FILE LAST UPDATED: 9 AUG 2006 <20060809/UP>
MOST RECENT DERWENT UPDATE: 200651 <200651/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

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http://www.stn-international.de/stndatabases/details/ipc_reform.html and
http://scientific.thomson.com/media/scpdf/ipcrdwpi.pdf <<<

>>> FOR FURTHER DETAILS ON THE FORTHCOMING DERWENT WORLD PATENTS INDEX ENHANCEMENTS PLEASE VISIT:
http://www.stn-international.de/stndatabases/details/dwpi_r.html <<<

FILE USPATFULL
FILE COVERS 1971 TO PATENT PUBLICATION DATE: 10 Aug 2006 (20060810/PD)
FILE LAST UPDATED: 10 Aug 2006 (20060810/ED)
HIGHEST GRANTED PATENT NUMBER: US7089595
HIGHEST APPLICATION PUBLICATION NUMBER: US2006179536
CA INDEXING IS CURRENT THROUGH 8 Aug 2006 (20060808/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 10 Aug 2006 (20060810/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Apr 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Apr 2006

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CA SUBSCRIBER PRICE	0.00	-39.75

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Inventor Search

=> d ibib abs l5 1-8

L5 ANSWER 1 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:660683 HCAPLUS
TITLE: A new "smart" polyelectrolyte drug carrier responsive to pH and glutathione for intracellular delivery of antisense oligonucleotides
AUTHOR(S): **Hoffman, Allan S.**; Bulmus, Volga; **Murthy, Niren**; **Stayton, Patrick S.**
CORPORATE SOURCE: Department of Bioengineering, University of Washington, Seattle, WA, 98195, USA
SOURCE: Abstracts of Papers, 228th ACS National Meeting, Philadelphia, PA, United States, August 22-26, 2004 (2004), POLY-221. American Chemical Society: Washington, D. C.
CODEN: 69FTZ8
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Cytoplasmic delivery of enzyme-susceptible biomol. drugs is one of the major limitations in many therapeutic strategies, such as gene and antisense therapy, and vaccine development. Development of better delivery systems that can enhance the endosomal escape of such biotherapeutics and thereby avoid degradation by lysosomal enzymes, is still a major goal of drug delivery scientists. Inspired by the action of pH-sensitive peptides in the protein coats of certain viruses to enable endosomal escape of their DNA or RNA cargoes [1], we have been designing, synthesizing and characterizing a family of novel pH-responsive polymers that can similarly enhance cytoplasmic delivery of enzyme-susceptible drugs such as DNA, RNA, antisense oligonucleotides (asODNs), proteins and peptides. [2-6] In this study, a novel functionalized monomer, pyridyl disulfide acrylate (PDSA), was synthesized and incorporated into an amphiphilic copolymer consisting of methacrylic acid and Bu acrylate, which resulted in a pH-sensitive, **membrane-disruptive** terpolymer with functional groups, that allow thiol-containing mols. to be readily conjugated. We conjugated a thiol-terminated asODN to the backbone via disulfide linkages. We also conjugated a cysteine-hexalysine peptide to the backbone via disulfide linkages, and then used the hexalysine groups to ionically-complex an asODN to the backbone.

L5 ANSWER 2 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:340027 HCAPLUS
DOCUMENT NUMBER: 140:117092
TITLE: Design and synthesis of pH-responsive polymeric carriers that target uptake and enhance the intracellular delivery of oligonucleotides
AUTHOR(S): **Murthy, Niren**; Campbell, Jean; Fausto, Nelson; **Hoffman, Allan S.**; **Stayton, Patrick S.**
CORPORATE SOURCE: Department of Bioengineering, University of Washington, Seattle, WA, 98195, USA
SOURCE: Journal of Controlled Release (2003), 89(3), 365-374
CODEN: JCREEC; ISSN: 0168-3659
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The delivery of biomol. therapeutics that function intracellularly remains a significant challenge in the field of biotechnol. In this report, a new family of polymeric drug carriers that combine cell targeting, a pH-responsive **membrane-disruptive** component, and serum-stabilizing polyethylene glycol (PEG) grafts, is shown to direct the

uptake and endosomal release of oligonucleotides in a primary hepatocyte cell line. These polymers are called encrypted polymers and are graft terpolymers that consist of a hydrophobic, **membrane-disruptive** backbone onto which hydrophilic PEG chains have been grafted through acid-degradable linker acetal linkages. In this report, the ability of the encrypted polymers to deliver rhodamine-labeled oligonucleotides or PEG-FITC (a model macromol. drug) (5 kDa) into the cytoplasm of hepatocytes was investigated by fluorescence microscopy. Two new encrypted polymer derivs. (polymers E2 and E3) were synthesized that contained lactose for targeting to hepatocytes. Polymer E2 also has PEG-FITC conjugated to it, as a model macromol. drug, and polymer E3 contains a pendant hexalysine moiety for complexing oligonucleotides. The results of the fluorescence microscopy expts. show that the encrypted polymers direct vesicular escape and efficiently deliver oligonucleotides and macromols. into the cytoplasm of hepatocytes.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:32877 HCAPLUS
DOCUMENT NUMBER: 138:226584
TITLE: Bioinspired pH-Responsive Polymers for the Intracellular Delivery of Biomolecular Drugs
AUTHOR(S): **Murthy, Niren**; Campbell, Jean; Fausto, Nelson; **Hoffman, Allan S.**; **Stayton, Patrick S.**
CORPORATE SOURCE: Department of Bioengineering and Department of Pathology, University of Washington, Seattle, WA, 98195, USA
SOURCE: Bioconjugate Chemistry (2003), 14(2), 412-419
CODEN: BCCHES; ISSN: 1043-1802
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biotechnol. and pharmaceutical industries have developed a wide variety of potential therapeutics based on the mols. of biol.: DNA, RNA, and proteins. While these therapeutics have tremendous potential, effectively formulating and delivering them have also been a widely recognized challenge. A variety of viruses and toxins have evolved multi-functional biomols. to solve this problem by directing cellular uptake and enhancing biomol. transport to the cytoplasm from the low pH endosomal compartment. In the study reported here, we have designed and synthesized bio-inspired, pH-responsive polymeric carriers, which we call "encrypted polymers", that mimic the multi-functional design of biol. These encrypted polymers target and direct cellular uptake, as well as enhance cytosolic delivery by disrupting endosomal membranes in a pH-dependent fashion. We show that the encrypted polymeric carriers significantly enhance the delivery of oligonucleotides and peptides to the cytoplasm of cultured macrophages, demonstrating the potential of this approach for delivery of biotherapeutics and vaccines.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:932156 HCAPLUS
DOCUMENT NUMBER: 137:206503
TITLE: pH-Sensitive polymers that enhance intracellular drug delivery in vivo
AUTHOR(S): Kyriakides, Themis R.; Cheung, Charles Y.; **Murthy, Niren**; Bornstein, Paul; **Stayton,**

Patrick S.; Hoffman, Allan S.
CORPORATE SOURCE: Department of Biochemistry, University of Washington, Seattle, WA, 98195, USA
SOURCE: Journal of Controlled Release (2002), 78(1-3), 295-303
CODEN: JCREEC; ISSN: 0168-3659
PUBLISHER: Elsevier Science Ireland Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Cytosolic delivery from endosomes is critical for those drugs that are susceptible to attack by lysosomal enzymes, such as DNA, RNA, oligonucleotides, proteins and peptides. Therefore, we have designed pH-sensitive, **membrane-disruptive** polymers to enhance the release of drugs from the acidic endosomal compartment to the cytoplasm. We have found that one polymer in particular, poly(propylacrylic acid) (PPAA), is very effective at **membrane disruption** at pHs below 6.5, based on hemolysis studies. PPAA also significantly enhances in vitro transfections of lipoplex formulations in cell culture, and does so in the presence of as much as 50% serum. In this study, we have extended our in vitro hemolysis and cell culture studies to an in vivo murine excisional wound healing model. A pilot study with a green fluorescent protein (GFP)-encoding plasmid indicated that injection of formulations containing PPAA into healing wounds resulted in increased GFP expression. Subsequently, by administering sense and antisense DNA for the angiogenesis inhibitor thrombospondin-2 (TSP2), we were able to alter the wound healing response in TSP2-null and wild type mice, resp. Our findings showed that when PPAA was added to lipoplex formulations, expression of TSP2 was enhanced in TSP2-null mice compared to control formulations. These results show that PPAA can enhance in vivo transfections and that inhibition of TSP2 expression may lead to improved wound healing. These results suggest that PPAA can provide significant improvements in the in vivo efficacy of drugs such as DNA.
REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 5 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2001:525957 HCAPLUS
DOCUMENT NUMBER: 135:127195
TITLE: Enhanced transport of therapeutic and diagnostic agents using **membrane disruptive** acid-sensitive polymers
INVENTOR(S): **Hoffman, Allan S.; Stayton, Patrick S.; Murthy, Niren**
PATENT ASSIGNEE(S): University of Washington, USA
SOURCE: PCT Int. Appl., 50 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001051092	A2	20010719	WO 2001-US356	20010105
WO 2001051092	A3	20011206		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU,

ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: US 2000-174893P P 20000107

AB Compns. and methods for transport or release of therapeutic and diagnostic agents, metabolites or other analytes from cells, compartments within cells, or through cell layers or barriers are described. The compns. include a membrane barrier transport enhancing agent and are usually administered in combination with an enhancer and/or exposure to stimuli to effect disruption or altered permeability, transport or release. In a preferred embodiment, the compns. include compds. which disrupt endosomal membranes in response to the low pH in the endosomes but which are relatively inactive toward cell membranes (at physiol. pH, but can become active toward cell membranes if the environment is acidified below pH 6.8), coupled directly or indirectly to a therapeutic or diagnostic agent. Other disruptive agents can also be used, responsive to stimuli and/or enhancers other than pH, such as light, elec. stimuli, electromagnetic stimuli, ultrasound, temperature, or combinations thereof. The compds. can be coupled by ionic, covalent or H bonds to an agent to be delivered or to a ligand which forms a complex with the agent to be delivered. Agents to be delivered can be therapeutic and/or diagnostic agents. Treatments which enhance delivery such as ultrasound, iontophoresis, and/or electrophoresis can also be used with the disrupting agents. For example, a terpolymer of dimethylaminoethyl methacrylate, Bu methacrylate, and styrene benzaldehyde was prepared for the **membrane-disruptive** backbone which was then PEGylated with thiol-terminated monofunctional or heterofunctional PEGs. The acid-degradable linkage was a p-aminobenzaldehyde acetal.

L5 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2000:208542 HCAPLUS
DOCUMENT NUMBER: 133:109744
TITLE: pH sensitive **membrane disruptive** PEGylated polycations
AUTHOR(S): **Murthy, Niren; Stayton, Patrick S. ; Hoffman, Allan S.**
CORPORATE SOURCE: Department of Bioengineering, University of Washington, Seattle, WA, 98195, USA
SOURCE: Polymer Preprints (American Chemical Society, Division of Polymer Chemistry) (2000), 41(1), 1010-1011
CODEN: ACPPAY; ISSN: 0032-3934
PUBLISHER: American Chemical Society, Division of Polymer Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A new method for the synthesis of novel PEGylated pH sensitive **membrane-disruptive** polycations as potential oligonucleotide delivery vehicles has been presented. The strategy is based on grafting PEG onto a hydrophobic-polycationic backbone through an acid degradable acetal linkage. The acetal linkage used for the PEGylation of Copolymer I had a half life of 15 min at pH 5.4, but at pH 7.4 less than 10% of the acetals were hydrolyzed after 80 min. Copolymer I has a hydrolysis rate suitable for drug delivery purposes. The hydrolysis of the PEG grafts and activation of its **membrane disruptive** activity occur in less than 20 min at pH 5.0. Copolymer I was **membrane disruptive** at pH 5.0 but not at pH 7.4. The above copolymers should therefore have applications for the delivery of neg. charged polyanions such as DNA or ODNs to cells.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:451212 HCAPLUS
DOCUMENT NUMBER: 131:106813
TITLE: Enhanced transport using **membrane disruptive** agents
INVENTOR(S): **Hoffman, Allan S.**; **Stayton, Patrick**; Press, Oliver; Tirrell, David; **Murthy, Niren**; Lackey, Chantal; Crum, Lawrence A.; Mourad, Pierre D.; Porter, Tyrone M.
PATENT ASSIGNEE(S): University of Washington, USA; University of Massachusetts
SOURCE: PCT Int. Appl., 54 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9934831	A1	19990715	WO 1999-US122	19990105
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2317549	AA	19990715	CA 1999-2317549	19990105
CA 2317549	C	20060411		
AU 9920261	A1	19990726	AU 1999-20261	19990105
AU 758368	B2	20030320		
EP 1044021	A1	20001018	EP 1999-900750	19990105
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 2001007666	A1	20010712	US 1999-226044	19990105
US 6835393	B2	20041228		
JP 2002500201	T2	20020108	JP 2000-527278	19990105
US 2005136102	A1	20050623	US 2004-857626	20040528

PRIORITY APPLN. INFO.:

US 1998-70411P	P	19980105
US 1999-226044	A1	19990105
WO 1999-US122	W	19990105

AB Compns. and methods for transport or release of therapeutic and diagnostic agents or metabolites or other analytes from cells, compartments within cells, or through cell layers or barriers are described. The compns. include a membrane barrier transport enhancing agent and are usually administered in combination with an enhancer and/or exposure to stimuli to effect disruption or altered permeability, transport or release. In a preferred embodiment, the compns. include compds. which disrupt endosomal membranes in response to the low pH in the endosomes but which are relatively inactive toward cell membranes, coupled directly or indirectly to a therapeutic or diagnostic agent. Other disruptive agents can also be used, responsive to stimuli and/or enhancers other than pH, such as light, elec. stimuli, electromagnetic stimuli, ultrasound, temperature, or combinations thereof. The compds. can be coupled by ionic, covalent or H bonds to an agent to be delivered or to a ligand which forms a complex with the agent to be delivered. Agents to be delivered can be therapeutic and/or diagnostic agents. Treatments which enhance delivery such as ultrasound, iontophoresis, and/or electrophoresis can also be used with the disrupting agents. The ability of the GALA peptide to lyse erythrocytes was compared with that of an GALA/poly(acrylic acid) conjugate at pH 5.0. The

conjugate gave 70% lysis at 100 μg.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 8 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:255413 HCAPLUS
DOCUMENT NUMBER: 131:78256
TITLE: Hemolytic Activity of pH-Responsive Polymer-Streptavidin Bioconjugates
AUTHOR(S): Lackey, Chantal A.; **Murthy, Niren**; Press, Oliver W.; Tirrell, David A.; **Hoffman, Allan S.**; **Stayton, Patrick S.**
CORPORATE SOURCE: Department of Bioengineering and Department of Medicine, University of Washington, Seattle, WA, 98195, USA
SOURCE: Bioconjugate Chemistry (1999), 10(3), 401-405
CODEN: BCCHES; ISSN: 1043-1802
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Drug delivery systems that increase the rate and/or quantity of drug release to the cytoplasm are needed to enhance cytosolic delivery and to circumvent nonproductive cell trafficking routes. We have previously demonstrated that poly(2-ethylacrylic acid) (PEAAc) has pH-dependent hemolytic properties, and more recently, we have found that poly(2-propylacrylic acid) (PPAAc) displays even greater pH-responsive hemolytic activity than PEAAc at the acidic pHs of the early endosome. Thus, these polymers could potentially serve as endosomal releasing agents in immunotoxin therapies. In this paper, we have investigated whether the pH-dependent **membrane disruptive** activity of PPAAc is retained after binding to a protein. We did this by measuring the hemolytic activity of PPAAc-streptavidin model complexes with different protein to polymer stoichiometries. Biotin was conjugated to amine-terminated PPAAc, which was subsequently bound to streptavidin by biotin complexation. The ability of these samples to disrupt red blood cell membranes was investigated for a range of polymer concns., a range of pH values, and two polymer-to-streptavidin ratios of 3:1 and 1:1. The results demonstrate that (a) the PPAAc-streptavidin complex retains the ability to lyse the RBC lipid bilayers at low pHs, such as those existing in endosomes, and (b) the hemolytic ability of the PPAAc-streptavidin complex is similar to that of the free PPAAc.
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT